

University of Dundee

Mitotic kinase anchoring proteins

Fulcher, Luke J.; Sapkota, Gopal P.

Published in:
Cell Cycle

DOI:
[10.1080/15384101.2020.1728014](https://doi.org/10.1080/15384101.2020.1728014)

Publication date:
2020

Document Version
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):

Fulcher, L. J., & Sapkota, G. P. (2020). Mitotic kinase anchoring proteins: the navigators of cell division. *Cell Cycle*, 19(5), 505-524. <https://doi.org/10.1080/15384101.2020.1728014>

General rights

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Mitotic kinase anchoring proteins: the navigators of cell division

Luke J Fulcher¹ and Gopal P Sapkota¹

¹Medical Research Council Protein Phosphorylation and Ubiquitylation Unit, University of Dundee, Dundee, Scotland, UK. Address correspondence to: l.fulcher@dundee.ac.uk; g.sapkota@dundee.ac.uk

Keywords: CDK, Aurora, PLK, mitosis, CK1 α , cell cycle

Abstract

The coordinated activities of many protein kinases, acting on multiple protein substrates, ensures the error-free progression through mitosis of eukaryotic cells. Enormous research effort has thus been devoted to studying the roles and regulation of these mitotic kinases, and to the identification of their physiological substrates. Central for the timely deployment of specific protein kinases to their appropriate substrates during the cell division cycle are the many anchoring proteins, which serve critical regulatory roles. Through direct association, anchoring proteins are capable of modulating the catalytic activity and/or sub-cellular distribution of the mitotic kinases they associate with. The key roles of some anchoring proteins in cell division are well-established, whilst others are still being unearthed. Here, we review the current knowledge on anchoring proteins for some mitotic kinases, and highlight how targeting anchoring proteins for inhibition, instead of the mitotic kinases themselves, could be advantageous for disrupting the cell division cycle.

Introduction

Accurate execution of the cell division cycle results in the precise duplication and subsequent separation of DNA and cytoplasm into two newly-formed daughter cells. The cell cycle is conventionally divided into two main phases: interphase, where the cell prepares for division, and M phase, where the cell divides into two genetically-identical daughter cells. Multiple regulatory checkpoints exist in cells to ensure that the cell cycle progresses with precision and accuracy, as errors at any point can be detrimental to the cell and organism as a whole (1-3). Indeed, many disease states, most notably cancer, have been linked to aberrant cell cycle control (2, 4, 5). Deciphering the regulatory nodes of the cell cycle is thus a topic of wide research interest, from both a basic science and therapeutic perspective.

Of the post-translational modifications known to regulate the cell cycle, protein phosphorylation constitutes one of the most studied to date. This phosphorylation-centred mitotic research focus likely stems from observations that the entry into mitosis is accompanied by a profound increase in the level of protein phosphorylation throughout the

cell (2, 6, 7). Historically, the observation that protein phosphorylation was dramatically increased following entry into mitosis was a key cornerstone in the identification of maturation promoting factor (MPF) – a cytoplasmic factor first identified in *Xenopus* oocytes, capable of stimulating entry into M-phase of the cell cycle (8, 9). MPF was later shown to consist of a protein kinase we now refer to as Cyclin-Dependent Kinase 1 (CDK1), and its associated regulatory subunit Cyclin (10). Since then, several other protein kinases have been found to have instrumental roles in eukaryotic cell division, and enormous effort has been devoted to understanding the roles of these protein kinases in mitosis, and to the identification of their physiological substrates. Indeed, many studies have determined the critical roles protein phosphorylation plays in key mitotic processes, including chromosome condensation and mitotic spindle assembly (11).

However, mitotic kinases require strict spatiotemporal regulation in order to exert their effects on the cell cycle. Indeed, many mitotic kinases exhibit dynamic changes in their subcellular distribution as mitosis progresses, or simultaneously reside at distinct mitotic structures such as centrosomes and kinetochores. The vital roles of anchoring proteins [defined in relation to other accessory binding proteins in Box 1 (12)] in these processes, acting to spatiotemporally coordinate mitotic kinase recruitment to key subcellular structures, and hence their substrates, are critically important for an accurate cell division. Here, we review the best-characterised anchoring proteins for some mitotic kinases, and summarise the mounting evidence supporting crucial roles for these diverse signalling proteins in coordinating the cell division cycle.

Cyclins control CDKs, the master regulators of the cell cycle

Cyclin-dependent kinases (CDKs) are a family of Ser/Thr protein kinases, whose catalytic activity depends on regulatory subunits termed Cyclins. This opening statement is a culmination of decades of research efforts that established that CDKs are indeed, as their name suggests, *Cyclin-dependent*. The identification of MPF (9) was a crucial first step in the subsequent determination of CDK-Cyclin interplay during mitosis. Although MPF was identified in 1971, the discovery of the MPF catalytic component did not come until 1988 when a purified MPF preparation from *Xenopus* oocytes was shown to contain two major proteins of 32 kDa and 45 kDa masses respectively (10). One year prior, homologs of the Cdc2 protein kinase were shown to be functionally conserved from yeast to humans (13): Cdc2 was originally identified in genetic screens searching for yeast mutants with defects in cell division (14, 15), and Cdc2 was later shown to be essential for cell-cycle progression (16). As Cdc2 was also a 32 kDa mitotic protein, it was speculated that the 32 kDa protein present in MPF purifications may be a *Xenopus* homolog of Cdc2. Consistent with this idea, an antibody recognising a conserved 16 amino acid sequence of Cdc2 was capable of depleting the purified MPF preparation of its MPF activity (17). Importantly, this antibody immunoprecipitated both

1 the 45 kDa and the 32 kDa proteins present in the MPF preparation (17), suggesting that Cdc2
2 and an associated protein may constitute the functional MPF complex.

3
4 Simultaneously, independent studies in sea urchin oocytes led to the identification of some
5 proteins that were synthesised and degraded at each cleavage division (18). Due to this
6 cyclical nature in their expression, these proteins were termed Cyclins. Subsequently, Cyclins
7 were cloned from fertilised clam embryos, and the ectopic introduction of Cyclin A mRNA was
8 shown to promote meiosis in *Xenopus* oocytes, suggesting that a rise in Cyclin A may drive
9 progression into M phase (19). The biochemical connection between Cdc2 and Cyclins came
10 in 1989 when researchers determined that Cdc2 associates with Cyclin A and B in starfish,
11 clam and *Xenopus* oocytes (20-22). Thus, Cdc2 thereafter became known as Cyclin-dependent
12 Kinase 1 (CDK1). At this time, it was also proposed that Cyclin proteolysis may drive
13 inactivation of the associated CDK (20), thereby promoting the idea that mitotic kinase
14 activity could be regulated by interacting proteins in cells.

15
16 With the advent of cDNA libraries and Polymerase Chain Reaction (PCR) technology, many
17 other CDK family members were identified, and their role as critical regulators of eukaryotic
18 cell division began to be fully appreciated (23-26). To date, 20 members of the CDK family
19 have been identified, and these have been designated CDK1-CDK20 (27). Decades of
20 molecular research have now determined that A-type Cyclins bind CDK1 and CDK2 and these
21 CDK-Cyclin A complexes act to resolve S phase and promote entry into the G2 phase (28).
22 During G2, A-type Cyclins are degraded through ubiquitin-mediated proteolysis, and the B-
23 type Cyclins are actively synthesised. Consequently, CDK1 associates with the newly-
24 translated Cyclin B and this active complex is thought to regulate several key steps during the
25 G2/M transition (11, 28). Many substrates have been reported for CDK1-Cyclin B during this
26 transition, including histones, whose phosphorylation by CDK1 promotes chromosome
27 condensation, and lamins, whose phosphorylation triggers nuclear envelope breakdown
28 (NEBD). Notably, CDK1-Cyclin B complexes have been shown to localise to centrosomes
29 during prophase, and phosphorylate the motor protein Eg5 in order to promote centrosome
30 separation (11). CDK1 has also been shown to activate several other mitotic protein kinases
31 (11, 28), and as such, CDK1 is often regarded as the master mitotic kinase. Furthermore, the
32 inactivation of CDK1-Cyclin B complexes is required for mitotic exit, and this inactivation is
33 achieved through the proteasomal degradation of Cyclin B following its ubiquitination by the
34 anaphase-promoting complex E3 ligase, leaving behind inactive, isolated CDK1 in the process
35 (1, 29).

36
37 Cyclins are also key mediators of CDK localisation during the cell cycle. In both humans and
38 *Xenopus*, Cyclin A, and thus the CDK1-Cyclin A complex, is found within the nucleus from S-
39 phase until the breakdown of the nuclear envelope (30, 31). Cyclin B and its associated CDK1
40 partner, on the other hand, is cytoplasmic in G2 and enters the nucleus just prior to NEBD
41 (30, 31). Cyclin B possesses a nuclear export signal, and this signal maintains Cyclin B in the

cytoplasm during interphase (32-35). Following NEBD, CDK1-Cyclin B is found on the spindle apparatus as well as on condensed chromosomes (30). These differences in Cyclin localisation are thought to underpin CDK1 substrate specificity *in vivo*. A pioneering study sought to determine whether it was the availability of a Cyclin within a subcellular compartment that was the limiting step in the cell cycle-dependent regulation of localised CDK activity. Expression of a Cyclin B1 mutant protein lacking its intrinsic nuclear export signal led to retention of Cyclin B1 in the nucleus, and this mutant protein was capable of stimulating DNA synthesis even in the absence of the native DNA synthesis-promoting Cyclin, Cyclin E (36). Thus, the spatial proximity and availability of Cyclins to CDKs appears to be critical for the spatiotemporal regulation of localised CDK activity. Phosphorylation of Cyclin B in prophase was found to regulate the nuclear translocation of Cyclin B in prophase, and a mitotic kinase termed Polo-like Kinase 1 (discussed in detail in the subsequent sections) was found to be the kinase responsible for this key mitotic event (37). Intriguingly, a related Cyclin termed Cyclin F was shown to be required for the nuclear translocation of Cyclin B, in a manner dependent on the intrinsic nuclear localisation signals of Cyclin F (38). This was the first example of a Cyclin-Cyclin protein interaction (38).

Interestingly, indicative of a potential role in regulating microtubule attachments or spindle assembly checkpoint signalling, Cyclin B1 was later found to reside at kinetochores (39, 40). Recently, two independent studies have highlighted the role of the spindle assembly checkpoint protein Mad1 in promoting CDK1-Cyclin B1 recruitment to kinetochores (41, 42). In stark contrast, the related B-type Cyclin, Cyclin B2, localises to the Golgi apparatus to enforce CDK1-mediated disassembly of this organelle during mitosis (43, 44). Interestingly, a chimeric protein composed of the N-terminus of Cyclin B2 and the C-terminus of Cyclin B1 was shown to associate with the Golgi apparatus, suggesting that the N-termini of Cyclins may determine their localisation patterns in cells (43). Importantly, this chimeric Cyclin did not show reduced binding to CDK1, nor did it affect CDK1 activation, when compared with wild-type Cyclins (43).

Future efforts aimed at determining the interacting partners of specific Cyclins may shed light on how these critical CDK1 regulators are controlled and recruited to specific subcellular compartments, where the associated CDKs act during the cell division cycle. However, it should be noted that Cyclin-interacting proteins are unlikely to fully explain the control of CDK1-Cyclin localisation. Indeed, phosphorylation of Cyclin B has been shown to be important for retaining Cyclin B in the cytoplasm (37, 45), and the binding of Cyclin B to the nuclear export regulating protein CRM1 (Chromosome Region Maintenance 1 protein homolog) has also been established as a key regulatory mechanism of Cyclin B nuclear translocation (32).

Anchoring proteins regulating Aurora kinases: an exemplar relationship

1 The *aurora* gene was first identified in a *Drosophila* screen aimed at identifying genes whose
2 products could control cell cycle progression (46, 47). With the discovery of homologs in other
3 species, the Aurora kinases have emerged as central players in cell division (48). Aurora
4 kinases are highly conserved, and present with a similar domain architecture between
5 homologs (49). Aurora kinases possess a Ser/Thr protein kinase domain sandwiched between
6 N- and C-terminal domains (50). The N- and C- terminal domains are thought to be important
7 in regulating Aurora kinase stability, as well as in determining the interaction partners of
8 distinct Aurora kinases (50). In humans, there are three Aurora kinases, designated AURKA, B
9 and C, and they display distinct subcellular distributions (11). AURKA localises to the
10 duplicated centrosomes at the start of S phase, and shifts to bipolar spindle microtubules
11 during mitosis (51, 52). AURKB localises to chromosomes in prophase and the centromere in
12 prometaphase, before shifting to the central spindle in anaphase and the mid-body in
13 cytokinesis (51, 52). The least-studied family member, AURKC, is localised to chromosomes
14 during mitosis and is thought to enhance AURKB function, but, unlike AURKA and B, is
15 principally expressed in the male and female germline of mammals (48, 53).

16
17 The ability of Aurora kinases to achieve such diverse localisation patterns during mitosis is
18 determined through the binding to different regulatory anchoring proteins. For example,
19 following NEBD, AURKA is recruited to spindle microtubules by binding the microtubule-
20 associated protein Targeting Protein for Xklp2 (TPX2), where TPX2 serves the additional
21 purpose of allosterically activating AURKA (48, 54, 55) (Figure 1A). TPX2 also contributes to
22 full AURKA activation by shielding it from inhibitory dephosphorylation by Protein
23 Phosphatase 1 (PP1) (56, 57). Crucially, TPX2 localisation to spindle microtubules is
24 independent of its interaction with AURKA, consistent with the idea that TPX2 recruits AURKA
25 to the spindle to regulate spindle assembly and spindle microtubule dynamics (58). Indeed,
26 the AURKA-TPX2 interaction was shown to be critically important for the assembly of spindles
27 of correct length, and for faithful chromosome segregation (58). Furthermore, the AURKA-
28 TPX2 interaction was shown to be important for AURKA stability (59). TPX2-silenced U2OS
29 cells showed lower AURKA protein levels in G2 and prometaphase, whereas overexpression
30 of the AURKA-binding domain of TPX2 blocked AURKA degradation in telophase (59). Adding
31 more complexity to the AURKA-TPX2 interaction, TPX2 itself appears to be an AURKA
32 substrate. In *Xenopus*, TPX2 was phosphorylated by AURKA (56, 60), and in HeLa cells AURKA-
33 phosphorylated TPX2 was shown to regulate spindle length (61).

34
35 AURKA also localises to centrosomes throughout the cell cycle. The localisation of AURKA to
36 centrosomes was shown to be dependent on the protein Bora (Figure 1A), a highly-conserved
37 AURKA-interacting protein originally identified based on phenotypic similarities - both AURKA
38 and Bora mutants exhibited identical centrosome maturation defects (62). The AURKA-Bora
39 complex was suggested to be the kinase complex responsible for phosphorylating and
40 activating PLK1 (63), a key mitotic kinase required for centrosome maturation and spindle
41 formation. Bora-depleted cells present with multipolar mitotic spindles - an effect

1 reminiscent of TPX2 knockdown (58). Although the recruitment of AURKA to centrosomes is
2 dependent on Bora, whether Bora achieves this in a direct or indirect manner remains to be
3 determined. Regardless, Bora is thought to be a key AURKA activator in cells (62). However,
4 it should be noted that Bora doesn't appear to enhance AURKA activity *per se*, but rather acts
5 to make the activation loop of PLK1 more accessible for phosphorylation by AURKA (64). Thus,
6 in the context of AURKA-dependent PLK1 phosphorylation, Bora can be considered an AURKA
7 activating protein, but the *in vitro* AURKA activity immunoprecipitated with Bora is far less
8 than the AURKA activity isolated in TPX2 immunoprecipitates (65). Once cells entered mitosis,
9 Bora was shown to be phosphorylated and degraded in a PLK1-dependent manner, and this
10 phosphodegradation event is thought to be a prerequisite for the recruitment of AURKA to spindle
11 microtubules by TPX2 (65) (Figure 1A).

12
13 AURKB, on the other hand, is a component of the Chromosome Passenger Complex (CPC),
14 which consists of three non-enzymatic AURKB-regulatory proteins termed INner CENTromere
15 Protein (INCENP), Borealin and Survivin (66-69) (Figure 1B). INCENP, Borealin and Survivin all
16 act as both targeting and activating subunits of AURKB (66-68), although the evidence in
17 favour of Survivin being a *bona fide* AURKB-activating protein remains controversial (70, 71).
18 Interestingly, formation of the CPC appears to be essential for the stability of individual
19 proteins within the complex (66, 70). The CPC, and hence AURKB activity, has been linked to
20 the correction of microtubule-chromosome attachment errors and activation of the spindle
21 assembly checkpoint (67).

22
23 As described earlier, the CPC exhibits a very dynamic localisation profile during cell division,
24 and this change in localisation is largely thought to coordinate AURKB activity towards its
25 substrates during mitosis. Importantly, when either INCENP, Survivin or Borealin are
26 mislocalised, the other complex members also mislocalise (66, 70, 72-74). At the onset of
27 mitosis, the CPC localises to chromosomes, and in prometaphase to inner centromeres (51,
28 52, 67). During the metaphase-to-anaphase transition, the CPC is found on central spindle
29 microtubules, before concentrating at the midbody during the latter stages of cell division
30 (51, 52, 67). INCENP acts as the platform on which the CPC is assembled, and the N-terminus
31 of INCENP mediates CPC recruitment to centromeres (75). Biochemical studies determined
32 that it was the first 58 amino acids of INCENP that are required for binding to Borealin and
33 Survivin, and are critical for the localisation of the CPC to the centromere, spindle midzone
34 and midbody (66, 75, 76). Crucially, this dynamic localisation profile correlates with the
35 pleiotropic functions of the CPC during cell division, and thus provides an elegant example of
36 how mitotic kinase anchoring proteins can facilitate the many functions of their associated
37 kinase partners, simply by coordinating the sub-cellular distribution of that kinase.
38 Interestingly, a single amino acid change in human AURKA (G198N – AURKB has an Asn at the
39 equivalent residue to G198) renders the kinase AURKB-like, promoting localisation to
40 chromosomes and interaction with INCENP and Survivin. Intriguingly, this AURKA mutant was
41 able to rescue mitotic defects resulting from AURKB knockdown (77, 78).

The importance of Survivin in localising the CPC to chromatin is well-established. Following phosphorylation of Histone H3 on Thr3 by the Ser/Thr protein kinase Haspin, Survivin binds phospho-Histone H3 (Thr3), and acts to recruit the CPC to chromosomes, where AURKB is activated and phosphorylates Histone H3 on Ser10 to regulate chromosome condensation (79) (Figure 1B). Crucially, Survivin was capable of binding phospho-Histone H3 (Thr3) in the absence of the other CPC proteins, strongly suggesting that Survivin is the CPC component that mediates CPC recruitment to chromatin (79). In late mitosis, when the Haspin site on Histone H3 is dephosphorylated, CPC localisation to chromatin is also suppressed, and thus this dephosphorylation event is thought to be key for the redistribution of the CPC as mitosis progresses (79-81). Curiously, whilst the majority of the CPC components exist in a quaternary complex to coordinate the functions of the CPC, a second complex consisting of solely AURKB and INCENP was proposed as an additional AURKB complex, functioning to regulate Histone H3 Ser10 phosphorylation (72). However, whether this sub-complex is merely an intermediate in the assembly of the full CPC cannot, at present, be ruled out.

During the metaphase-to-anaphase transition, the CPC relocates from the inner centromere to central spindle microtubules. This localisation event is associated with a decrease in CDK1 activity, and is dependent on both AURKB and phosphatase catalytic activity (82-84). Mechanistically, the kinesin-6 protein MKLP2 (Mitotic Kinesin-like Protein 2), which binds central spindle microtubules, associates with INCENP and AURKB to direct the CPC to the central spindle (82, 85, 86) (Figure 1B). This interaction only occurs in anaphase, once the inhibitory CDK1-mediated phosphorylation of MKLP2 is reduced (82) (Figure 1B). Interestingly, MKLP2 also binds and directs the phosphatase Cdc14A to central spindle microtubules, and Cdc14A has been proposed to dephosphorylate INCENP to promote to relocation of the CPC to the central spindle in anaphase (85).

The Polo Box Domain – a unique mode for mitotic kinase recruitment

Polo-like Kinases (PLKs) are a family of Ser/Thr protein kinases that were first described in lower eukaryotes (87, 88). In *Drosophila melanogaster*, a mutant of the protein Polo was found to present with defects in mitosis (88), and PLK homologs were subsequently identified in mammals (89). All PLKs have a similar domain architecture, with an N-terminal kinase domain, and a C-terminal regulatory Polo Box domain containing two signature motifs termed “Polo boxes” (89) (Figure 2A). Humans have 5 PLK enzymes termed PLK1-5, however the exact role and contribution of each PLK isoform is not well understood (89). As PLK1 is largely thought to mediate most of the mitotic functions attributed to the *D. melanogaster* Polo, the major focus of the subsequent section will be on PLK1. However, key roles for the related kinase PLK4 in centriole duplication have also been reported in recent years, and as such, the mitotic functions of PLK4 will also be discussed.

1 Similar to many other protein kinases, human PLK1 is activated through phosphorylation
2 within its T-loop (at Thr210), by an upstream protein kinase (90-92). As described earlier, the
3 AURKA-Bora complex has been reported to phosphorylate and activate PLK1 in cells (63)
4 (Figure 2B), although other upstream kinases have also been implicated (93). Emerging as a
5 key regulatory feature of PLK1 in cells, the polo boxes have been shown to be instrumental in
6 mediating PLK localisation (94-97). The Polo-box Domain (PBD) of PLK1 acts as a
7 phosphopeptide-binding motif, and in this capacity, the PBD binds specific phosphorylated
8 proteins to facilitate the recruitment of PLK1 to those proteins. The priming phosphorylation
9 event on the target protein, which generates the phosphoepitope onto which the PBD binds,
10 can be mediated by different protein kinases, allowing for wider crosstalk between different
11 kinases during mitosis, but is most frequently performed by CDK1 or PLK1 itself (98-101)
12 (Figure 2B). In cases where the phosphorylated PLK1-anchoring proteins are localised to
13 cellular structures, such as kinetochores and centrosomes, PBD-mediated binding also
14 imparts spatial control on PLK1 (102-105). To date, many such PBD-binding proteins have
15 been identified (106). In the absence of a phosphoepitope to bind to, the PBD is thought to
16 associate with the kinase domain of PLK1, thereby impeding its kinase activation and
17 substrate binding (99). However, upon PBD-phosphoepitope association, the kinase domain
18 is thought to be released from the PBD, and together with T-loop phosphorylation by
19 upstream kinases, PLK1 achieves maximal activation. Simultaneously, the PBD-
20 phosphoepitope association determines where within the cell PLK1 is localised, and hence
21 where its activity is utilised (99)

22
23 In both interphase and mitosis, PLK1 localises to centrosomes, and centrosome-localised PLK1
24 in mitosis has been reported to be critical for spindle pole formation, and positioning of the
25 mitotic spindle (107, 108). Interestingly, following inhibition of PLK1, monopolar spindles
26 form due to defective centrosome separation, and cells arrest in mitosis (107, 108). Whilst
27 the exact PLK1-dependent substrate landscape is not fully elucidated, many proteins involved
28 in centrosome function and microtubule dynamics have been reported to be PLK1 targets
29 (106, 109). Recently, the pericentriolar material-localised protein Gravin was shown to be a
30 PLK1 anchoring protein in cells (110) (Figure 2C). *In vitro* studies suggested that PLK1
31 associated with Gravin only when Gravin was phosphorylated on Thr766 (111), suggesting a
32 canonical PBD-mediated mode of interaction. Loss of Gravin in primary prostate cancer cells
33 was associated with elevated micronuclei formation, and a redistribution of active PLK1 to
34 different protein complexes within centrosomes (110). Interestingly, Gravin was also shown
35 to interact with AURKA (112), suggesting that Gravin may act to streamline AURKA-PLK1
36 signalling at centrosomes, similar to the Bora protein discussed earlier (Figure 2C). The Gravin-
37 PLK1-AURKA complex was shown to be down-regulated in human testicular seminoma (112),
38 suggesting that disruption of this signalling axis may contribute to the disease. In a similar
39 vein, it is also interesting to note that the centrosome-localised coiled-coil protein Cep192
40 was also shown to act as a scaffold protein for both PLK1 and AURKA, and this macromolecular
41 complex was critical for centrosome maturation (113) (Figure 2C). However, the

1 phosphoepitope-generating residue of Cep192, Thr46, that facilitates PLK1 binding through
2 its PBD, does not conform to a CDK1 consensus motif and is thought to be phosphorylated by
3 PLK1 itself (113). As Gravin and Cep192 appear to act in similar capacities, it will be interesting
4 to decipher the centrosomal PLK1/AURKA substrates that are dependent on, and unique to,
5 each anchoring protein. Interestingly, the mother centriole-associated protein Cenexin has
6 also been shown to associate with PLK1, following phosphorylation of Cenexin at Ser796 by
7 CDK1 (114) (Figure 2C). The PLK1-Cenexin interaction was shown to be required for
8 recruitment of pericentriolar material proteins, and thus maturation of centrosomes (114).

9
10 In mitotic cells undergoing the metaphase-to-anaphase transition, PLK1 also localises to
11 kinetochores, the centromere-associated protein complexes to which microtubules attach
12 (89, 102). Kinetochores can sense when K-fibre microtubules are unattached to
13 chromosomes, and even a single unattached chromosome can trigger a checkpoint
14 mechanism known as the Spindle Assembly Checkpoint (SAC) (115). The localisation of PLK1
15 to kinetochores thus suggests a role for PLK1 in kinetochore assembly, regulation of
16 kinetochore-microtubule connections, and/or modulation of the SAC. A number of
17 kinetochore-localised proteins have been implicated in the recruitment of PLK1 to
18 kinetochores. For example, the SAC protein Bub1 was shown to bind PLK1 in a CDK1-
19 dependent manner, and depletion of Bub1 with siRNA was accompanied with a reduction in
20 PLK1 at kinetochores (116) (Figure 2D). In line with this, overexpression of wild-type Bub1,
21 but not the Bub1-T609A mutant that cannot be phosphorylated by CDK1, restored PLK1
22 localisation in Bub1-silenced cells (116). Additionally, the outer kinetochore-associated
23 protein CLIP-170 (Cytoplasmic Linker Protein 170) was shown to be required for PLK1
24 kinetochore association, again following CDK1-mediated phosphorylation of CLIP-170 to
25 promote the PLK1-CLIP-170 interaction (117) (Figure 2D). Depletion of CLIP-170 resulted in
26 chromosome congression defects and kinetochore microtubule instability (117). The dynein-
27 associated protein dynactin was also found to facilitate PLK1 targeting to kinetochores, again
28 in a CDK1-dependent manner (118) (Figure 2D). Thus, the recurring theme in the kinetochore
29 recruitment of PLK1 appears to be CDK1-mediated phosphorylation of a target protein that
30 then recruits PLK1 through PBD binding. However, whether this holds true for all PLK1-
31 recruiting proteins remains to be determined. Indeed, PLK1 is also reported to be localised to
32 the midbody (119). As CDK1-Cyclin B activity would be abolished at this stage of mitosis, there
33 is scope for other mitotic kinases, and for PLK1 itself, in promoting PBD-dependent
34 recruitment of PLK1 at the latter stages of cell division.

35
36 During anaphase, PLK1 also localises to central spindle microtubules. The PLK1 anchoring
37 protein PRC1 (Protein Regulating Cytokinesis 1), which facilitates PLK1 recruitment to the
38 central spindle, was discovered in a proteomic screen of anaphase-arrested HeLa cells (120)
39 (Figure 2E). At this point in mitosis CDK1 activity is vastly reduced compared to the CDK1
40 activity present in metaphase, so it was speculated that CDK1 was not the kinase responsible
41 for generating the phosphoepitope on PRC1 to promote PLK1 binding. Indeed, it was

determined that PLK1 itself creates the phosphoepitope on PRC1, by phosphorylating Thr602 (120). Interestingly, CDK1 appears to phosphorylate PRC1 on Thr470 and Thr481 in the earlier stages of mitosis, to inhibit the PLK1-PRC1 interaction until anaphase (120) (Figure 2E). The kinesin-6 protein MKLP2, discussed earlier in the context of recruitment of the CPC to central spindle microtubules, also appears to direct PLK1 to the central spindle in anaphase, following its phosphorylation on Ser528 by PLK1 (Figure 2E) (101). Thus, in the latter stages of mitosis when CDK1 activity is diminished, the importance of PLK1 in generating the phosphoepitope on its anchoring proteins to facilitate its PBD binding appears to be much greater.

The vast array of diverse PLK1 anchoring proteins raises the question of why the cell has evolved so many diverse PBD-binding proteins. Is it that PLK1 activity at centrosomes, kinetochores and the central spindle is required at spatially distinct protein complexes within those mitotic structures? Or is there simply more to the PBD-substrate association relationship, and each PLK1 anchoring protein is also a PLK1 substrate whose phosphorylation by PLK1 is critical for centrosome and spindle dynamics? Regardless, targeting PLK1 anchoring proteins in specific mitotic contexts would allow the disruption of selective PLK1 mitotic functions, whilst targeting PLK1 kinase activity would be predicted to disrupt all PLK1-dependent mitotic processes. Such a targeted approach may prove powerful when researching the biology of specific PLK1-containing protein complexes.

In the case of PLK4, two centrosomal scaffold proteins, Cep152 and Cep192, have been shown to be required for PLK4 recruitment to centrosomes, as well as for correct centriole duplication (121, 122). PLK4 differs from PLK1 in that PLK4 harbours a single polo-box at its C-terminus, in addition to a so-called cryptic PBD within the central region of the protein (123). This cryptic PBD, composed of two pseudo-polo boxes (123), is both necessary and sufficient for PLK4 targeting to centrosomes, and has been proposed to mediate the interaction between PLK4 and its binding partners (123, 124). Indeed, it was the cryptic PBD that was found to bind both Cep192 and Cep152 in a competitive manner, in order to regulate the recruitment of PLK4 to centrosomes (121, 122). Interestingly, both the PLK4-Cep192 and PLK4-Cep152 interactions were shown to be regulated in a spatiotemporal manner (121). However, the molecular basis for these interactions remains to be determined. That said, it is clear that PLK4 does not adopt the phosphoepitope-binding mechanism that PLK1 employs to bind its targets. It will be interesting to determine how this cryptic PBD mediates PLK4 recruitment, and if there are more PLK4 anchoring proteins within the centrosome or other mitotic sites.

Assembling the Mitotic Checkpoint Complex: all eyes on KNL1

Named by Lester Sharp in the 1930s, kinetochores are the power-generating business-ends of chromosomes during mitosis (125). In their capacity to bridge spindle microtubules to chromosomes, kinetochores are key focal points of phosphorylation-mediated regulation, for

1 both the SAC and cell cycle progression (126, 127). The kinases CDK1, PLK1 and AURKB have
2 all been implicated in the regulation of the SAC and subsequent attachment error correction
3 (127). However, other kinases have critical roles in the SAC, which are discussed further
4 herein.

5
6 The transition to anaphase is triggered by the E3 ligase Anaphase-promoting
7 Complex/Cyclosome (APC/C), which acts to ubiquitinate inhibitors of mitotic exit (Cyclin B)
8 and of chromosome segregation (Securin), thereby marking them for proteolysis (29, 128).
9 Thus, when an attachment error is created, the SAC acts to inhibit the APC/C, and in doing so,
10 prevents the metaphase-to-anaphase transition. The kinetochore-localised multi-protein
11 complex that is responsible for the inhibition of APC/C in response to attachment error is
12 called the mitotic checkpoint complex (MCC) (127). The MCC assembles on unattached
13 kinetochores, and following its assembly, is free to diffuse throughout the cell to inhibit the
14 APC/C (127). MCC assembly is coordinated by the kinase monopolar spindle 1 (Mps1), and
15 Mps1 activity drives the recruitment of SAC proteins such as the kinase budding uninhibited
16 by benzimidazoles 1 (Bub1), the regulatory proteins Bub3, mitotic arrest-deficient 1 (Mad1),
17 Mad2, and the pseudokinase Bub-related 1 (BubR1) (129-132). Mps1 thus acts as the master
18 regulator of the SAC.

19
20 Mps1 is activated by autophosphorylation upon its localisation to kinetochores, which is
21 regulated by AURKB activity, again illustrating some of the crosstalk evident between mitotic
22 kinases (133-135). Mps1 is then in a prime position to efficiently recruit the MCC, including
23 the Bub1 kinase. The importance of Bub1 is perhaps best showcased in experiments in yeast,
24 where deletion of *BUB1* in *S. pombe* increased the rate of chromosome missegregation, and
25 deletion of *BUB1* in *S. cerevisiae* caused slow growth and chromosome loss (136, 137). Bub1
26 is also required for the kinetochore localisation of Mad1 and Mad2, following its recruitment
27 by Mps1 (138).

28
29 Central to the coordinated MCC assembly on unattached kinetochores is the scaffold protein
30 KNL1 (Kinetochore Scaffold 1) (Figure 3). Through a variety of conserved functional domains
31 and motifs (139), KNL1 essentially acts as the SAC assembly platform. Following
32 phosphorylation of KNL1 by Mps1 (Figure 3A), Bub1 and Bub3 directly associate with KNL1
33 (140-143), and subsequently Bub1 acts to recruit Mad1, Mad2 and BubR1 to the kinetochore
34 (138, 144) (Figure 3B). Thus, loss of KNL1 disrupts the localisation of all SAC proteins, with the
35 exception of Mps1. Crucially, disruption of Mps1-dependent KNL1 phosphorylation
36 attenuates the binding of Bub1 and Bub3 to KNL1, and is accompanied with chromosome
37 congression and SAC signalling defects (141-143). Mechanistically, Mps1 phosphorylates so-
38 called MELT repeats in KNL1, and these phosphoepitopes facilitate the binding to Bub1 and
39 Bub3. Bub1 then acts as a scaffold to assemble the remainder of the SAC proteins. Thus,
40 despite being a kinase, the major role of Bub1 in the SAC appears to be its protein anchoring
41 function, and not its catalytic activity. However, Bub1 has been linked to the phosphorylation

1 and subsequent inhibition of Cdc20, thereby providing a potential mechanistic insight into
2 how the SAC acts to inhibit the APC/C following chromosome attachment error (145, 146). In
3 line with such a central role in SAC signalling, KNL1 depletion in HeLa cells was shown to
4 disrupt SAC-induced mitotic arrest following exposure to microtubule poisons (147). KNL1 is
5 also required for silencing of the SAC signal to enable the metaphase-to-anaphase transition
6 once all kinetochores are engaged, although this is beyond the scope of this review and has
7 been reviewed elsewhere (148).

9 **Delivery of a pleiotropic kinase to the mitotic spindle: CK1 α sets the bar**

11 The Casein Kinase 1 (CK1) family forms its own distinct branch of the kinome tree (149), and
12 constitutes one of the first Ser/Thr protein kinase families to be discovered (150). The CK1
13 branch includes the CK1 isoforms, and the closely-related Vaccinia-related kinases (VRKs), and
14 Tau Tubulin Kinase 1 (TTBK1) members (149, 151). To date, six mammalian CK1 isoforms,
15 namely α , γ 1, γ 2, γ 3, δ and ϵ , and their associated splice variants, have been reported based
16 on their high degree of homology within the kinase domains (152-154).

18 The absence of isoform-selective CK1 inhibitors has led to confusion regarding exactly which
19 CK1 isoform represents the physiological kinase for each of the identified substrates. The
20 kinase domains of CK1 isoforms (sequence-wise and structurally) are very similar, and all CK1
21 isoforms are thought to be constitutively active *in vitro*, capable of phosphorylating substrate
22 residues conforming to identical motifs (152-154). Due to most cellular proteins harbouring
23 at least one CK1 consensus phospho-motif, it is perhaps not surprising that hundreds of CK1
24 substrates have been reported. Added complexity arises when considering the cellular
25 environment. Indeed, the substrate specificity of CK1 isoforms *in vitro* is thought to be largely
26 different from that observed *in vivo*, and different isoforms are known to impact distinct
27 biological processes, suggesting tight regulation of distinct isoforms in cells (152-154). This
28 difference in the *in vitro* versus *in vivo* substrate specificity is attributed to intracellular
29 regulatory mechanisms involved in modulating CK1 isoforms, such as functional binding
30 partners and post-translational modifications. Furthermore, as the kinase domain of CK1
31 isoforms constitutes the vast majority of the protein sequence, regulatory domains that are
32 prevalent in many other kinases are very small, if not completely absent, in CK1 isoforms. This
33 adds further merit to the need for additional regulatory CK1-binding partners in cells. These
34 attributes have prompted researchers to ascertain the precise molecular mechanisms by
35 which the activities of specific CK1 isoforms towards their cellular substrates are governed.

37 The *S. cerevisiae* orthologue of CK1 was among the first kinases identified to have a role in
38 the regulation of cell cycle progression (155). In mammals however, where there are multiple
39 CK1 isoforms present, the precise contribution of each isoform to the regulation of the cell
40 division cycle is not well understood. CK1 δ has been found on centrosomes, and displays high
41 affinity towards microtubules in response to DNA damage, suggesting a possible checkpoint

1 role for CK1 δ in cell division (156, 157). Furthermore, inhibition of CK1 δ/ϵ using the CK1 δ/ϵ -
2 specific inhibitor IC261 is accompanied by cell cycle arrest (156). In addition to CK1 δ , CK1 α
3 has long been suggested to have a role in mitosis. Early immunostaining efforts identified
4 CK1 α on mitotic spindles (158), and morpholino-mediated knockdown of CK1 α triggered
5 mitotic arrest and chromosomal alignment defects in mouse oocytes (159). However, CK1 α
6 has also been implicated in many other, diverse signalling processes (Figure 4A).

7
8 Recently, the FAM83 family of proteins have emerged as central regulators of CK1 isoforms
9 in cells (160-162). In the context of cell division, the selective CK1 α -binding protein FAM83D
10 was shown to be absolutely required for CK1 α to localise to mitotic spindles (163) (Figure 4B).
11 Cells devoid of *FAM83D*, or those harbouring a CK1-binding deficient F283A mutant of
12 FAM83D, failed to recruit CK1 α to the spindle apparatus (163). Concomitantly, these cells
13 presented with spindle misorientation phenotypes, and exhibited a delay in the metaphase-
14 to-anaphase transition (163). CK1 α was shown to regulate the process of spindle positioning,
15 which is critical for both accurate development and maintenance of healthy adult tissues, in
16 a manner dependent on its delivery to the spindle by FAM83D (163). As CK1 α is known to
17 regulate many diverse signalling processes, from Wnt signalling to circadian rhythm (153,
18 164), targeting FAM83D had the added benefit of impacting only one CK1 α -specific process,
19 whilst seemingly not affecting other aspects of CK1 α biology – a feat impossible to achieve
20 with CK1 α knockdown or its inhibition with small molecules (Figure 4C). Indeed, the gene
21 encoding CK1 α , *CSNK1A1*, appears to be an essential gene required for cell viability (165), and
22 the fact that *FAM83D* knockout cells are viable would argue that the essential CK1 α functions
23 remain intact following FAM83D ablation.

24
25 Interestingly, a recent phosphoproteomic study reported that roughly 50% of all cell cycle-
26 regulated phosphopeptides conform to the predicted CK1 consensus substrate motifs (6).
27 Thus, whilst CK1 α is only beginning to be considered as a mitotic kinase, there is huge scope
28 for future efforts aimed at identifying the physiological CK1 α substrates in mitosis, and
29 deciphering how CK1 α exerts its regulation on spindle positioning at the molecular level. It is
30 also noteworthy that, like many cell-cycle regulated anchoring proteins such as Cyclins and
31 TPX2 (18, 166), *FAM83D* transcripts and protein levels are regulated during the cell cycle, with
32 levels rising during mitosis and falling upon mitotic exit, whereas those of CK1 α remain
33 unchanged throughout (163).

34
35 FAM83D itself relies on the non-motor, microtubule-associated protein HMMR (aka RHAMM)
36 for its recruitment to spindle microtubules (108, 163). Thus, cells devoid of *HMMR* phenocopy
37 cells devoid of *FAM83D*, and present with spindle positioning defects (108, 163). This model
38 would therefore suggest that CK1 α requires both FAM83D and HMMR for its spindle
39 localisation, and therefore its mitotic function. Why would the cell evolve such a two-pronged
40 mechanism to recruit CK1 α to spindles? Whilst this question still requires some work in terms
41 of mechanistic and structural insights, it is interesting to note that HMMR has also been

reported to direct the TPX2-AURKA complex to spindle microtubules in mitosis (55). HMMR-silenced cells presented with a reduction in both TPX2 and active AURKA at mitotic spindles, and could not establish spindles of the correct length (55) – a phenotype common to cells in which the AURKA-TPX2 interaction is compromised (58). Mechanistically, HMMR is thought to participate in the Ran-GTP-dependent microtubule nucleation pathway, and serves to anchor NEDD1 (Neural precursor cell Expressed Developmentally Down-regulated protein 1) to promote TPX2-AURKA recruitment to sites of microtubule assembly, where AURKA phosphorylates NEDD1 on Ser405 (167-169). PLK1-dependent phosphorylation of NEDD1 regulates the recruitment of NEDD1 (170, 171), and HMMR in turn regulates PLK1 activity (108), implying the potential existence of an HMMR-dependent feedback loop to coordinate spindle assembly. The AURKA- and CK1 α -containing HMMR complexes are likely distinct, as neither TPX2 nor AURKA were detected in endogenous FAM83D immunoprecipitations subjected to mass spectrometric analysis (163). Thus, it may transpire that HMMR acts as a central scaffolding protein to which anchoring proteins like FAM83D and TPX2 associate in order to direct their associated kinase cargos to distinct locations along the mitotic spindle.

Conclusions and future perspectives

Here, we set out to review the diverse modes of regulation bestowed on mitotic kinases by anchoring proteins. Through the regulation of protein kinase localisation, activity, stability and/or substrate accessibility, mitotic kinase anchoring proteins have evolved as critical regulators of mitosis in cells. Whilst research has primarily focussed on the roles and regulation of the conventional mitotic kinase families mentioned above, it is beginning to be appreciated that these kinase families alone cannot account for the full extent of protein phosphorylation that is evident during mitosis (6, 7, 172, 173). Interestingly, a recent phosphoproteomic study sought to identify and assign cell cycle-regulated phosphopeptides to known kinases, based on the optimal consensus motifs present within the phosphopeptides (6). Whilst CDKs and PLKs were identified within the top ten kinase families, the vast majority of phosphopeptides conformed to the predicted motifs for CK1, Casein kinase 2 (CK2), Protein Kinase A (PKA) and Glycogen Synthase Kinase 3 (GSK3) (6). Whilst predicted motifs do not necessarily point to direct roles of the aforementioned kinases per se, it is interesting to note that mitotic roles for some of these kinases, and others such as Protein Kinase C (PKC) and the NIMA-related kinases (NEKs), have already been reported (174-177). In some cases, these kinases have been shown to localise to key mitotic sites, such as the spindle apparatus and kinetochore. For example, CK2 α was shown to localise to mitotic spindles in both a phosphorylation and PIN1 (Peptidyl-prolyl cis-trans Isomerase NIMA-interacting 1)-dependent manner (178).

Pleiotropic kinases, which are active throughout the cell cycle, are often more challenging to study compared with their pure mitotic kinase counterparts, as their inhibition or knockdown will have consequences beyond just their mitotic functions. In this context, the identification

of specific anchoring proteins that underpin their mitotic roles will be of paramount importance in understanding precisely how these kinases contribute to the regulation of the cell division cycle. Indeed, taking the FAM83D-CK1 α interaction mentioned earlier as an example, being able to manipulate FAM83D-bound CK1 α , rather than pan-cellular CK1 α activity, afforded the key advantage of allowing the selective investigation into the role of CK1 α in cell division, whilst seemingly unaffected the non-mitotic CK1 α functions (163). Uncovering key anchoring proteins that control the subcellular distribution and/or activities of protein kinases during mitosis will undoubtedly hold promise for differentiating their mitotic roles from their other non-mitotic functions. Furthermore, in cases such as CDKs, Aurora kinases, PLK1, and CK1 α , for which there are multiple anchoring proteins already identified, being able to target each anchoring protein in turn will shed light on complex-specific functions of these key mitotic kinases.

The coordinated cross-talk evident between mitotic kinases, acting in concert to orchestrate a successful cell division, highlights the importance of kinase signalling networks in driving the cell cycle. Critical for the success of such finely-tuned cross-talk events are the many anchoring proteins that serve to spatiotemporally direct the mitotic kinases to their correct sites of action. For example, and as discussed above, Bora serves to direct AURKA to centrosomes, where the AURKA-Bora complex activates PLK1, leading to a whole plethora of downstream responses, including the degradation of Bora through PLK1-mediated Bora phosphorylation. As a direct consequence, AURKA, now no longer bound to Bora, is free to bind TPX2 on spindle microtubules and regulate spindle microtubule dynamics. Furthermore, coupling Histone H3 dephosphorylation and reduced CDK1 activity to the redistribution of the CPC from chromatin to the central spindle in anaphase, is a great example of how cooperative phosphorylation events can regulate the subcellular localisation of anchoring proteins, and hence the associated mitotic kinase. Another example stems from the inhibitory phosphorylation of PRC1 by CDK1 in the earlier stages of mitosis, to inhibit the PRC1-PLK1 interaction until anaphase when CDK1 activity is reduced.

Anchoring proteins can also facilitate mitotic kinase cross-talk by acting as the physical bridge between two or more mitotic kinases and their substrates. In the case of Gravin and Cep192, these anchoring proteins can bind both PLK1 and AURKA, suggesting that perhaps Gravin and Cep192 require phosphorylation inputs from both PLK1 and AURKA. Alternatively, Gravin and Cep192 may act to localise both PLK1 and AURKA in proximity to common substrates, that function in response to dual phosphorylation from both kinases to coordinate centrosome maturation. Furthermore, if the latter hypothesis is correct, such phosphorylation inputs do not necessarily need to be synergistic. These phosphorylation events could be antagonistic or hierarchical, in order to finetune the downstream biology. In the case of HMMR, although there is no evidence that HMMR can simultaneously bind both the FAM83D-CK1 α and TPX2-AURKA complexes, the sheer spatial proximity of two HMMR complexes (one containing

HMMR-FAM83D-CK1 α and the other containing HMMR-TPX2-AURKA) on the spindle, may be sufficient to allow cross-talk between CK1 α and AURKA on shared substrates.

Given such evident cross-talk between mitotic kinases, targeting mitotic kinase anchoring proteins for inhibition may lead to disruption of entire mitotic signalling networks, as opposed to the ablation of a single specific mitotic kinase complex and its associated function. As such, anchoring proteins present themselves as novel therapeutic targets in disease. Recent years have seen the development of so-called PROteolysis TARgeting Chimeras (PROTACs), which serve to degrade proteins of interest within the cell by directing them to endogenous E3 ubiquitin ligase machinery (179, 180). The fact that some mitotic kinase anchoring proteins, such as Cyclins and FAM83D, are quickly turned over following mitotic exit, whereas their interacting kinases are not, not only provides insights into their importance in controlling protein kinase function during mitosis, but suggest that their premature proteolysis may act as an effective means of inhibiting mitotic kinase function. Clearly, such information can be harnessed to target key anchoring proteins for degradation prior to mitosis, which may facilitate effective disruption of the associated kinase function during mitosis. Such strategies potentially offer alternative opportunities for therapeutic intervention in diseases such as cancer, in which control of the cell cycle is compromised.

Acknowledgements:

We thank K. Wu and A. Saurin for their highly-appreciated critical reading of this manuscript, and for their suggestions. We would like to apologise to the authors of any relevant studies that we may have inadvertently missed out in this review.

Declaration of Interests:

The authors declare that they have no competing interests.

References:

1. King RW, Deshaies RJ, Peters JM, Kirschner MW. How proteolysis drives the cell cycle. *Science*. 1996;274(5293):1652-9.
2. Dephoure N, Zhou C, Villen J, Beausoleil SA, Bakalarski CE, Elledge SJ, et al. A quantitative atlas of mitotic phosphorylation. *Proc Natl Acad Sci U S A*. 2008;105(31):10762-7.
3. Kronja I, Orr-Weaver TL. Translational regulation of the cell cycle: when, where, how and why? *Philos Trans R Soc Lond B Biol Sci*. 2011;366(1584):3638-52.
4. Massague J. G1 cell-cycle control and cancer. *Nature*. 2004;432(7015):298-306.
5. Williams GH, Stoeber K. The cell cycle and cancer. *J Pathol*. 2012;226(2):352-64.
6. Ly T, Whigham A, Clarke R, Brenes-Murillo AJ, Estes B, Madhessian D, et al. Proteomic analysis of cell cycle progression in asynchronous cultures, including mitotic subphases, using PRIMMUS. *Elife*. 2017;6.

7. Nousiainen M, Sillje HH, Sauer G, Nigg EA, Korner R. Phosphoproteome analysis of the human mitotic spindle. *Proc Natl Acad Sci U S A*. 2006;103(14):5391-6.
8. Rao PN, Johnson RT. Mammalian cell fusion: studies on the regulation of DNA synthesis and mitosis. *Nature*. 1970;225(5228):159-64.
9. Masui Y, Markert CL. Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *J Exp Zool*. 1971;177(2):129-45.
10. Lohka MJ, Hayes MK, Maller JL. Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. *Proc Natl Acad Sci U S A*. 1988;85(9):3009-13.
11. Nigg EA. Mitotic kinases as regulators of cell division and its checkpoints. *Nat Rev Mol Cell Biol*. 2001;2(1):21-32.
12. Buday L, Tompa P. Functional classification of scaffold proteins and related molecules. *FEBS J*. 2010;277(21):4348-55.
13. Lee MG, Nurse P. Complementation used to clone a human homologue of the fission yeast cell cycle control gene *cdc2*. *Nature*. 1987;327(6117):31-5.
14. Hartwell LH. *Saccharomyces cerevisiae* cell cycle. *Bacteriol Rev*. 1974;38(2):164-98.
15. Hartwell LH, Mortimer RK, Culotti J, Culotti M. Genetic Control of the Cell Division Cycle in Yeast: V. Genetic Analysis of *cdc* Mutants. *Genetics*. 1973;74(2):267-86.
16. Russell P, Nurse P. *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*: a look at yeasts divided. *Cell*. 1986;45(6):781-2.
17. Gautier J, Norbury C, Lohka M, Nurse P, Maller J. Purified Maturation-Promoting Factor Contains the Product of a *Xenopus* Homolog of the Fission Yeast-Cell Cycle Control Gene *Cdc2+*. *Cell*. 1988;54(3):433-9.
18. Evans T, Rosenthal ET, Youngblom J, Distel D, Hunt T. Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell*. 1983;33(2):389-96.
19. Swenson KI, Farrell KM, Ruderman JV. The clam embryo protein cyclin A induces entry into M phase and the resumption of meiosis in *Xenopus* oocytes. *Cell*. 1986;47(6):861-70.
20. Draetta G, Luca F, Westendorf J, Brizuela L, Ruderman J, Beach D. Cdc2 Protein-Kinase Is Complexed with Both Cyclin-a and Cyclin-B - Evidence for Proteolytic Inactivation of Mpf. *Cell*. 1989;56(5):829-38.
21. Booher RN, Alfa CE, Hyams JS, Beach DH. The Fission Yeast Cdc2 Cdc13 Suc1 Protein-Kinase - Regulation of Catalytic Activity and Nuclear-Localization. *Cell*. 1989;58(3):485-97.
22. Meijer L, Arion D, Golsteyn R, Pines J, Brizuela L, Hunt T, et al. Cyclin Is a Component of the Sea-Urchin Egg M-Phase Specific Histone H-1 Kinase. *Embo Journal*. 1989;8(8):2275-82.
23. Elledge SJ, Spottswood MR. A new human p34 protein kinase, CDK2, identified by complementation of a *cdc28* mutation in *Saccharomyces cerevisiae*, is a homolog of *Xenopus* Eg1. *EMBO J*. 1991;10(9):2653-9.
24. Xiong Y, Zhang H, Beach D. D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. *Cell*. 1992;71(3):505-14.
25. Meyerson M, Enders GH, Wu CL, Su LK, Gorka C, Nelson C, et al. A family of human *cdc2*-related protein kinases. *EMBO J*. 1992;11(8):2909-17.
26. Matsushime H, Ewen ME, Strom DK, Kato JY, Hanks SK, Roussel MF, et al. Identification and properties of an atypical catalytic subunit (p34PSK-J3/cdk4) for mammalian D type G1 cyclins. *Cell*. 1992;71(2):323-34.

27. Malumbres M, Harlow E, Hunt T, Hunter T, Lahti JM, Manning G, et al. Cyclin-dependent kinases: a family portrait. *Nat Cell Biol.* 2009;11(11):1275-6.
28. Malumbres M, Barbacid M. Mammalian cyclin-dependent kinases. *Trends Biochem Sci.* 2005;30(11):630-41.
29. Chang DC, Xu N, Luo KQ. Degradation of cyclin B is required for the onset of anaphase in Mammalian cells. *J Biol Chem.* 2003;278(39):37865-73.
30. Pines J, Hunter T. Human cyclins A and B1 are differentially located in the cell and undergo cell cycle-dependent nuclear transport. *J Cell Biol.* 1991;115(1):1-17.
31. Minshull J, Golsteyn R, Hill CS, Hunt T. The A- and B-type cyclin associated cdc2 kinases in *Xenopus* turn on and off at different times in the cell cycle. *EMBO J.* 1990;9(9):2865-75.
32. Yang J, Bardes ES, Moore JD, Brennan J, Powers MA, Kornbluth S. Control of cyclin B1 localization through regulated binding of the nuclear export factor CRM1. *Genes Dev.* 1998;12(14):2131-43.
33. Toyoshima F, Moriguchi T, Wada A, Fukuda M, Nishida E. Nuclear export of cyclin B1 and its possible role in the DNA damage-induced G2 checkpoint. *EMBO J.* 1998;17(10):2728-35.
34. Hagting A, Karlsson C, Clute P, Jackman M, Pines J. MPF localization is controlled by nuclear export. *EMBO J.* 1998;17(14):4127-38.
35. Moore JD, Yang J, Truant R, Kornbluth S. Nuclear import of Cdk/cyclin complexes: identification of distinct mechanisms for import of Cdk2/cyclin E and Cdc2/cyclin B1. *J Cell Biol.* 1999;144(2):213-24.
36. Moore JD, Kirk JA, Hunt T. Unmasking the S-phase-promoting potential of cyclin B1. *Science.* 2003;300(5621):987-90.
37. Toyoshima-Morimoto F, Taniguchi E, Shinya N, Iwamatsu A, Nishida E. Polo-like kinase 1 phosphorylates cyclin B1 and targets it to the nucleus during prophase. *Nature.* 2001;410(6825):215-20.
38. Kong M, Barnes EA, Ollendorff V, Donoghue DJ. Cyclin F regulates the nuclear localization of cyclin B1 through a cyclin-cyclin interaction. *EMBO J.* 2000;19(6):1378-88.
39. Bentley AM, Normand G, Hoyt J, King RW. Distinct sequence elements of cyclin B1 promote localization to chromatin, centrosomes, and kinetochores during mitosis. *Mol Biol Cell.* 2007;18(12):4847-58.
40. Chen Q, Zhang X, Jiang Q, Clarke PR, Zhang C. Cyclin B1 is localized to unattached kinetochores and contributes to efficient microtubule attachment and proper chromosome alignment during mitosis. *Cell Res.* 2008;18(2):268-80.
41. Alfonso-Perez T, Hayward D, Holder J, Gruneberg U, Barr FA. MAD1-dependent recruitment of CDK1-CCNB1 to kinetochores promotes spindle checkpoint signaling. *J Cell Biol.* 2019;218(4):1108-17.
42. Allan L, Reis M, Liu Y, Huis in 't Veld P, Kops GJPL, Musacchio A, et al. Cyclin B1 scaffolds MAD1 at the corona to activate the spindle assembly checkpoint. *BioRxiv.* 2019.
43. Draviam VM, Orrechia S, Lowe M, Pardi R, Pines J. The localization of human cyclins B1 and B2 determines CDK1 substrate specificity and neither enzyme requires MEK to disassemble the Golgi apparatus. *J Cell Biol.* 2001;152(5):945-58.
44. Jackman M, Firth M, Pines J. Human cyclins B1 and B2 are localized to strikingly different structures: B1 to microtubules, B2 primarily to the Golgi apparatus. *EMBO J.* 1995;14(8):1646-54.

45. Li J, Meyer AN, Donoghue DJ. Nuclear localization of cyclin B1 mediates its biological activity and is regulated by phosphorylation. *Proc Natl Acad Sci U S A*. 1997;94(2):502-7.
46. Glover DM. Mitosis in *Drosophila*. *J Cell Sci*. 1989;92 (Pt 2):137-46.
47. Glover DM, Leibowitz MH, McLean DA, Parry H. Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. *Cell*. 1995;81(1):95-105.
48. Carmena M, Ruchaud S, Earnshaw WC. Making the Auroras glow: regulation of Aurora A and B kinase function by interacting proteins. *Curr Opin Cell Biol*. 2009;21(6):796-805.
49. Ke YW, Dou Z, Zhang J, Yao XB. Function and regulation of Aurora/Ipl1p kinase family in cell division. *Cell Res*. 2003;13(2):69-81.
50. Bolanos-Garcia VM. Aurora kinases. *Int J Biochem Cell Biol*. 2005;37(8):1572-7.
51. Giet R, Prigent C. Aurora/Ipl1p-related kinases, a new oncogenic family of mitotic serine-threonine kinases. *J Cell Sci*. 1999;112 (Pt 21):3591-601.
52. Bischoff JR, Plowman GD. The Aurora/Ipl1p kinase family: regulators of chromosome segregation and cytokinesis. *Trends Cell Biol*. 1999;9(11):454-9.
53. Wang G, Jiang Q, Zhang C. The role of mitotic kinases in coupling the centrosome cycle with the assembly of the mitotic spindle. *J Cell Sci*. 2014;127(Pt 19):4111-22.
54. Kufer TA, Sillje HH, Korner R, Gruss OJ, Meraldi P, Nigg EA. Human TPX2 is required for targeting Aurora-A kinase to the spindle. *J Cell Biol*. 2002;158(4):617-23.
55. Chen H, Mohan P, Jiang J, Nemirovsky O, He D, Fleisch MC, et al. Spatial regulation of Aurora A activity during mitotic spindle assembly requires RHAMM to correctly localize TPX2. *Cell Cycle*. 2014;13(14):2248-61.
56. Evers PA, Erikson E, Chen LG, Maller JL. A novel mechanism for activation of the protein kinase Aurora A. *Curr Biol*. 2003;13(8):691-7.
57. Bayliss R, Sardon T, Vernos I, Conti E. Structural basis of Aurora-A activation by TPX2 at the mitotic spindle. *Mol Cell*. 2003;12(4):851-62.
58. Bird AW, Hyman AA. Building a spindle of the correct length in human cells requires the interaction between TPX2 and Aurora A. *J Cell Biol*. 2008;182(2):289-300.
59. Giubettini M, Asteriti IA, Scrofani J, De Luca M, Lindon C, Lavia P, et al. Control of Aurora-A stability through interaction with TPX2. *J Cell Sci*. 2011;124(Pt 1):113-22.
60. Evers PA, Maller JL. Regulation of *Xenopus* Aurora A activation by TPX2. *J Biol Chem*. 2004;279(10):9008-15.
61. Fu J, Bian M, Xin G, Deng Z, Luo J, Guo X, et al. TPX2 phosphorylation maintains metaphase spindle length by regulating microtubule flux. *J Cell Biol*. 2015;210(3):373-83.
62. Hutterer A, Berdnik D, Wirtz-Peitz F, Zigman M, Schleiffer A, Knoblich JA. Mitotic activation of the kinase Aurora-A requires its binding partner Bora. *Dev Cell*. 2006;11(2):147-57.
63. Macurek L, Lindqvist A, Lim D, Lampson MA, Klompaker R, Freire R, et al. Polo-like kinase-1 is activated by aurora A to promote checkpoint recovery. *Nature*. 2008;455(7209):119-23.
64. Seki A, Coppinger JA, Jang CY, Yates JR, Fang G. Bora and the kinase Aurora a cooperatively activate the kinase Plk1 and control mitotic entry. *Science*. 2008;320(5883):1655-8.
65. Chan EH, Santamaria A, Sillje HH, Nigg EA. Plk1 regulates mitotic Aurora A function through betaTrCP-dependent degradation of hBora. *Chromosoma*. 2008;117(5):457-69.

66. Vader G, Kauw JJ, Medema RH, Lens SM. Survivin mediates targeting of the chromosomal passenger complex to the centromere and midbody. *EMBO Rep.* 2006;7(1):85-92.
67. Vader G, Medema RH, Lens SM. The chromosomal passenger complex: guiding Aurora-B through mitosis. *J Cell Biol.* 2006;173(6):833-7.
68. Kelly AE, Sampath SC, Maniar TA, Woo EM, Chait BT, Funabiki H. Chromosomal enrichment and activation of the aurora B pathway are coupled to spatially regulate spindle assembly. *Dev Cell.* 2007;12(1):31-43.
69. Sessa F, Mapelli M, Ciferri C, Tarricone C, Areces LB, Schneider TR, et al. Mechanism of Aurora B activation by INCENP and inhibition by hesperadin. *Mol Cell.* 2005;18(3):379-91.
70. Honda R, Korner R, Nigg EA. Exploring the functional interactions between Aurora B, INCENP, and survivin in mitosis. *Mol Biol Cell.* 2003;14(8):3325-41.
71. Yue Z, Carvalho A, Xu Z, Yuan X, Cardinale S, Ribeiro S, et al. Deconstructing Survivin: comprehensive genetic analysis of Survivin function by conditional knockout in a vertebrate cell line. *J Cell Biol.* 2008;183(2):279-96.
72. Gassmann R, Carvalho A, Henzing AJ, Ruchaud S, Hudson DF, Honda R, et al. Borealin: a novel chromosomal passenger required for stability of the bipolar mitotic spindle. *J Cell Biol.* 2004;166(2):179-91.
73. Adams RR, Maiato H, Earnshaw WC, Carmena M. Essential roles of Drosophila inner centromere protein (INCENP) and aurora B in histone H3 phosphorylation, metaphase chromosome alignment, kinetochore disjunction, and chromosome segregation. *J Cell Biol.* 2001;153(4):865-80.
74. Carvalho A, Carmena M, Sambade C, Earnshaw WC, Wheatley SP. Survivin is required for stable checkpoint activation in taxol-treated HeLa cells. *J Cell Sci.* 2003;116(Pt 14):2987-98.
75. Ainsztein AM, Kandels-Lewis SE, Mackay AM, Earnshaw WC. INCENP centromere and spindle targeting: identification of essential conserved motifs and involvement of heterochromatin protein HP1. *J Cell Biol.* 1998;143(7):1763-74.
76. Klein UR, Nigg EA, Gruneberg U. Centromere targeting of the chromosomal passenger complex requires a ternary subcomplex of Borealin, Survivin, and the N-terminal domain of INCENP. *Mol Biol Cell.* 2006;17(6):2547-58.
77. Fu J, Bian M, Liu J, Jiang Q, Zhang C. A single amino acid change converts Aurora-A into Aurora-B-like kinase in terms of partner specificity and cellular function. *Proc Natl Acad Sci U S A.* 2009;106(17):6939-44.
78. Hans F, Skoufias DA, Dimitrov S, Margolis RL. Molecular distinctions between Aurora A and B: a single residue change transforms Aurora A into correctly localized and functional Aurora B. *Mol Biol Cell.* 2009;20(15):3491-502.
79. Kelly AE, Ghenoiu C, Xue JZ, Zierhut C, Kimura H, Funabiki H. Survivin reads phosphorylated histone H3 threonine 3 to activate the mitotic kinase Aurora B. *Science.* 2010;330(6001):235-9.
80. Qian J, Lesage B, Beullens M, Van Eynde A, Bollen M. PP1/Repo-man dephosphorylates mitotic histone H3 at T3 and regulates chromosomal aurora B targeting. *Curr Biol.* 2011;21(9):766-73.
81. Vagnarelli P, Ribeiro S, Sennels L, Sanchez-Pulido L, de Lima Alves F, Verheyen T, et al. Repo-Man coordinates chromosomal reorganization with nuclear envelope reassembly during mitotic exit. *Dev Cell.* 2011;21(2):328-42.

- 1 82. Hummer S, Mayer TU. Cdk1 negatively regulates midzone localization of the mitotic
2 kinesin Mklp2 and the chromosomal passenger complex. *Curr Biol.* 2009;19(7):607-12.
- 3 83. Pereira G, Schiebel E. Separase regulates INCENP-Aurora B anaphase spindle
4 function through Cdc14. *Science.* 2003;302(5653):2120-4.
- 5 84. Xu Z, Ogawa H, Vagnarelli P, Bergmann JH, Hudson DF, Ruchaud S, et al. INCENP-
6 aurora B interactions modulate kinase activity and chromosome passenger complex
7 localization. *J Cell Biol.* 2009;187(5):637-53.
- 8 85. Gruneberg U, Neef R, Honda R, Nigg EA, Barr FA. Relocation of Aurora B from
9 centromeres to the central spindle at the metaphase to anaphase transition requires MKlp2.
10 *J Cell Biol.* 2004;166(2):167-72.
- 11 86. Cesario JM, Jang JK, Redding B, Shah N, Rahman T, McKim KS. Kinesin 6 family
12 member Subito participates in mitotic spindle assembly and interacts with mitotic
13 regulators. *J Cell Sci.* 2006;119(Pt 22):4770-80.
- 14 87. Llamazares S, Moreira A, Tavares A, Girdham C, Spruce BA, Gonzalez C, et al. polo
15 encodes a protein kinase homolog required for mitosis in *Drosophila*. *Genes Dev.*
16 1991;5(12A):2153-65.
- 17 88. Sunkel CE, Glover DM. polo, a mitotic mutant of *Drosophila* displaying abnormal
18 spindle poles. *J Cell Sci.* 1988;89 (Pt 1):25-38.
- 19 89. Barr FA, Sillje HH, Nigg EA. Polo-like kinases and the orchestration of cell division.
20 *Nat Rev Mol Cell Biol.* 2004;5(6):429-40.
- 21 90. Qian YW, Erikson E, Maller JL. Mitotic effects of a constitutively active mutant of the
22 *Xenopus* polo-like kinase Plx1. *Mol Cell Biol.* 1999;19(12):8625-32.
- 23 91. Jang YJ, Ma S, Terada Y, Erikson RL. Phosphorylation of threonine 210 and the role of
24 serine 137 in the regulation of mammalian polo-like kinase. *J Biol Chem.*
25 2002;277(46):44115-20.
- 26 92. Lee KS, Erikson RL. Plk is a functional homolog of *Saccharomyces cerevisiae* Cdc5,
27 and elevated Plk activity induces multiple septation structures. *Mol Cell Biol.*
28 1997;17(6):3408-17.
- 29 93. Ji JH, Hwang HI, Lee HJ, Hyun SY, Kang HJ, Jang YJ. Purification and proteomic
30 identification of putative upstream regulators of polo-like kinase-1 from mitotic cell
31 extracts. *FEBS Lett.* 2010;584(20):4299-305.
- 32 94. Jang YJ, Lin CY, Ma S, Erikson RL. Functional studies on the role of the C-terminal
33 domain of mammalian polo-like kinase. *Proc Natl Acad Sci U S A.* 2002;99(4):1984-9.
- 34 95. Lee KS, Grenfell TZ, Yarm FR, Erikson RL. Mutation of the polo-box disrupts
35 localization and mitotic functions of the mammalian polo kinase Plk. *Proc Natl Acad Sci U S*
36 *A.* 1998;95(16):9301-6.
- 37 96. Song S, Grenfell TZ, Garfield S, Erikson RL, Lee KS. Essential function of the polo box
38 of Cdc5 in subcellular localization and induction of cytokinetic structures. *Mol Cell Biol.*
39 2000;20(1):286-98.
- 40 97. Reynolds N, Ohkura H. Polo boxes form a single functional domain that mediates
41 interactions with multiple proteins in fission yeast polo kinase. *J Cell Sci.* 2003;116(Pt
42 7):1377-87.
- 43 98. Elia AE, Cantley LC, Yaffe MB. Proteomic screen finds pSer/pThr-binding domain
44 localizing Plk1 to mitotic substrates. *Science.* 2003;299(5610):1228-31.
- 45 99. Elia AE, Rellos P, Haire LF, Chao JW, Ivins FJ, Hoepker K, et al. The molecular basis for
46 phosphodependent substrate targeting and regulation of Plks by the Polo-box domain. *Cell.*
47 2003;115(1):83-95.

- 1 100. Cheng KY, Lowe ED, Sinclair J, Nigg EA, Johnson LN. The crystal structure of the
2 human polo-like kinase-1 polo box domain and its phospho-peptide complex. *EMBO J.*
3 2003;22(21):5757-68.
- 4 101. Neef R, Preisinger C, Sutcliffe J, Kopajtich R, Nigg EA, Mayer TU, et al.
5 Phosphorylation of mitotic kinesin-like protein 2 by polo-like kinase 1 is required for
6 cytokinesis. *J Cell Biol.* 2003;162(5):863-75.
- 7 102. Arnaud L, Pines J, Nigg EA. GFP tagging reveals human Polo-like kinase 1 at the
8 kinetochore/centromere region of mitotic chromosomes. *Chromosoma.* 1998;107(6-7):424-
9 9.
- 10 103. Seong YS, Kamijo K, Lee JS, Fernandez E, Kuriyama R, Miki T, et al. A spindle
11 checkpoint arrest and a cytokinesis failure by the dominant-negative polo-box domain of
12 Plk1 in U-2 OS cells. *J Biol Chem.* 2002;277(35):32282-93.
- 13 104. Lee KS, Yuan YL, Kuriyama R, Erikson RL. Plk is an M-phase-specific protein kinase
14 and interacts with a kinesin-like protein, CHO1/MKLP-1. *Mol Cell Biol.* 1995;15(12):7143-51.
- 15 105. Golsteyn RM, Mundt KE, Fry AM, Nigg EA. Cell cycle regulation of the activity and
16 subcellular localization of Plk1, a human protein kinase implicated in mitotic spindle
17 function. *J Cell Biol.* 1995;129(6):1617-28.
- 18 106. Park JE, Soung NK, Johmura Y, Kang YH, Liao C, Lee KH, et al. Polo-box domain: a
19 versatile mediator of polo-like kinase function. *Cell Mol Life Sci.* 2010;67(12):1957-70.
- 20 107. Kiyomitsu T, Cheeseman IM. Chromosome- and spindle-pole-derived signals
21 generate an intrinsic code for spindle position and orientation. *Nat Cell Biol.* 2012;14(3):311-
22 7.
- 23 108. Connell M, Chen H, Jiang J, Kuan CW, Fotovati A, Chu TL, et al. HMMR acts in the
24 PLK1-dependent spindle positioning pathway and supports neural development. *Elife.*
25 2017;6.
- 26 109. Santamaria A, Wang B, Elowe S, Malik R, Zhang F, Bauer M, et al. The Plk1-
27 dependent phosphoproteome of the early mitotic spindle. *Mol Cell Proteomics.*
28 2011;10(1):M110 004457.
- 29 110. Colicino EG, Garrastegui AM, Freshour J, Santra P, Post DE, Kotula L, et al. Gravin
30 regulates centrosome function through PLK1. *Mol Biol Cell.* 2018;29(5):532-41.
- 31 111. Canton DA, Keene CD, Swinney K, Langeberg LK, Nguyen V, Pelletier L, et al. Gravin is
32 a transitory effector of polo-like kinase 1 during cell division. *Mol Cell.* 2012;48(4):547-59.
- 33 112. Hehnlly H, Canton D, Bucko P, Langeberg LK, Ogier L, Gelman I, et al. A mitotic kinase
34 scaffold depleted in testicular seminomas impacts spindle orientation in germ line stem
35 cells. *Elife.* 2015;4:e09384.
- 36 113. Joukov V, Walter JC, De Nicolo A. The Cep192-organized aurora A-Plk1 cascade is
37 essential for centrosome cycle and bipolar spindle assembly. *Mol Cell.* 2014;55(4):578-91.
- 38 114. Soung NK, Kang YH, Kim K, Kamijo K, Yoon H, Seong YS, et al. Requirement of
39 hCenexin for proper mitotic functions of polo-like kinase 1 at the centrosomes. *Mol Cell Biol.*
40 2006;26(22):8316-35.
- 41 115. Musacchio A. The Molecular Biology of Spindle Assembly Checkpoint Signaling
42 Dynamics. *Curr Biol.* 2015;25(20):R1002-18.
- 43 116. Qi W, Tang Z, Yu H. Phosphorylation- and polo-box-dependent binding of Plk1 to
44 Bub1 is required for the kinetochore localization of Plk1. *Mol Biol Cell.* 2006;17(8):3705-16.
- 45 117. Amin MA, Itoh G, Iemura K, Ikeda M, Tanaka K. CLIP-170 recruits PLK1 to
46 kinetochores during early mitosis for chromosome alignment. *J Cell Sci.* 2014;127(Pt
47 13):2818-24.

118. Yeh TY, Kowalska AK, Scipioni BR, Cheong FK, Zheng M, Derewenda U, et al. Dynactin helps target Polo-like kinase 1 to kinetochores via its left-handed beta-helical p27 subunit. *EMBO J.* 2013;32(7):1023-35.
119. Colicino EG, Hehnlly H. Regulating a key mitotic regulator, polo-like kinase 1 (PLK1). *Cytoskeleton (Hoboken).* 2018;75(11):481-94.
120. Neef R, Gruneberg U, Kopajtich R, Li X, Nigg EA, Sillje H, et al. Choice of Plk1 docking partners during mitosis and cytokinesis is controlled by the activation state of Cdk1. *Nat Cell Biol.* 2007;9(4):436-44.
121. Kim TS, Park JE, Shukla A, Choi S, Murugan RN, Lee JH, et al. Hierarchical recruitment of Plk4 and regulation of centriole biogenesis by two centrosomal scaffolds, Cep192 and Cep152. *Proc Natl Acad Sci U S A.* 2013;110(50):E4849-57.
122. Sonnen KF, Gabryjonczyk AM, Anselm E, Stierhof YD, Nigg EA. Human Cep192 and Cep152 cooperate in Plk4 recruitment and centriole duplication. *J Cell Sci.* 2013;126(Pt 14):3223-33.
123. Slevin LK, Nye J, Pinkerton DC, Buster DW, Rogers GC, Slep KC. The structure of the plk4 cryptic polo box reveals two tandem polo boxes required for centriole duplication. *Structure.* 2012;20(11):1905-17.
124. Habedanck R, Stierhof YD, Wilkinson CJ, Nigg EA. The Polo kinase Plk4 functions in centriole duplication. *Nat Cell Biol.* 2005;7(11):1140-6.
125. Sharp LW. Introduction to cytology. 3d ed. New York and London,: McGraw-Hill book company, inc.; 1934. xiv, 567 p. p.
126. London N, Biggins S. Signalling dynamics in the spindle checkpoint response. *Nat Rev Mol Cell Biol.* 2014;15(11):736-47.
127. Sacristan C, Kops GJ. Joined at the hip: kinetochores, microtubules, and spindle assembly checkpoint signaling. *Trends Cell Biol.* 2015;25(1):21-8.
128. Pines J. Cubism and the cell cycle: the many faces of the APC/C. *Nat Rev Mol Cell Biol.* 2011;12(7):427-38.
129. Nijenhuis W, von Castelmur E, Littler D, De Marco V, Tromer E, Vleugel M, et al. A TPR domain-containing N-terminal module of MPS1 is required for its kinetochore localization by Aurora B. *J Cell Biol.* 2013;201(2):217-31.
130. Kemmler S, Stach M, Knapp M, Ortiz J, Pfannstiel J, Ruppert T, et al. Mimicking Ndc80 phosphorylation triggers spindle assembly checkpoint signalling. *EMBO J.* 2009;28(8):1099-110.
131. Murray AW. A brief history of error. *Nat Cell Biol.* 2011;13(10):1178-82.
132. Vleugel M, Hoogendoorn E, Snel B, Kops GJ. Evolution and function of the mitotic checkpoint. *Dev Cell.* 2012;23(2):239-50.
133. Saurin AT, van der Waal MS, Medema RH, Lens SM, Kops GJ. Aurora B potentiates Mps1 activation to ensure rapid checkpoint establishment at the onset of mitosis. *Nat Commun.* 2011;2:316.
134. Santaguida S, Vernieri C, Villa F, Ciliberto A, Musacchio A. Evidence that Aurora B is implicated in spindle checkpoint signalling independently of error correction. *EMBO J.* 2011;30(8):1508-19.
135. Kang J, Chen Y, Zhao Y, Yu H. Autophosphorylation-dependent activation of human Mps1 is required for the spindle checkpoint. *Proc Natl Acad Sci U S A.* 2007;104(51):20232-7.

136. Bernard P, Hardwick K, Javerzat JP. Fission yeast bub1 is a mitotic centromere protein essential for the spindle checkpoint and the preservation of correct ploidy through mitosis. *J Cell Biol.* 1998;143(7):1775-87.
137. Warren CD, Brady DM, Johnston RC, Hanna JS, Hardwick KG, Spencer FA. Distinct chromosome segregation roles for spindle checkpoint proteins. *Mol Biol Cell.* 2002;13(9):3029-41.
138. Klebig C, Korinth D, Meraldi P. Bub1 regulates chromosome segregation in a kinetochore-independent manner. *J Cell Biol.* 2009;185(5):841-58.
139. Ghongane P, Kapanidou M, Asghar A, Elowe S, Bolanos-Garcia VM. The dynamic protein Knl1 - a kinetochore rendezvous. *J Cell Sci.* 2014;127(Pt 16):3415-23.
140. Primorac I, Weir JR, Chiroli E, Gross F, Hoffmann I, van Gerwen S, et al. Bub3 reads phosphorylated MELT repeats to promote spindle assembly checkpoint signaling. *Elife.* 2013;2:e01030.
141. Shepperd LA, Meadows JC, Sochaj AM, Lancaster TC, Zou J, Buttrick GJ, et al. Phosphodependent recruitment of Bub1 and Bub3 to Spc7/KNL1 by Mph1 kinase maintains the spindle checkpoint. *Curr Biol.* 2012;22(10):891-9.
142. London N, Ceto S, Ranish JA, Biggins S. Phosphoregulation of Spc105 by Mps1 and PP1 regulates Bub1 localization to kinetochores. *Curr Biol.* 2012;22(10):900-6.
143. Yamagishi Y, Yang CH, Tanno Y, Watanabe Y. MPS1/Mph1 phosphorylates the kinetochore protein KNL1/Spc7 to recruit SAC components. *Nat Cell Biol.* 2012;14(7):746-52.
144. Sharp-Baker H, Chen RH. Spindle checkpoint protein Bub1 is required for kinetochore localization of Mad1, Mad2, Bub3, and CENP-E, independently of its kinase activity. *J Cell Biol.* 2001;153(6):1239-50.
145. Reddy SK, Rape M, Margansky WA, Kirschner MW. Ubiquitination by the anaphase-promoting complex drives spindle checkpoint inactivation. *Nature.* 2007;446(7138):921-5.
146. Tang Z, Shu H, Oncel D, Chen S, Yu H. Phosphorylation of Cdc20 by Bub1 provides a catalytic mechanism for APC/C inhibition by the spindle checkpoint. *Mol Cell.* 2004;16(3):387-97.
147. Kiyomitsu T, Obuse C, Yanagida M. Human Blinkin/AF15q14 is required for chromosome alignment and the mitotic checkpoint through direct interaction with Bub1 and BubR1. *Dev Cell.* 2007;13(5):663-76.
148. Caldas GV, DeLuca JG. KNL1: bringing order to the kinetochore. *Chromosoma.* 2014;123(3):169-81.
149. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. *Science.* 2002;298(5600):1912-34.
150. Kumar R, Tao M. Multiple forms of casein kinase from rabbit erythrocytes. *Biochim Biophys Acta.* 1975;410(1):87-98.
151. Ikezu S, Ikezu T. Tau-tubulin kinase. *Front Mol Neurosci.* 2014;7:33.
152. Gross SD, Anderson RA. Casein kinase I: spatial organization and positioning of a multifunctional protein kinase family. *Cell Signal.* 1998;10(10):699-711.
153. Knippschild U, Gocht A, Wolff S, Huber N, Lohler J, Stoter M. The casein kinase 1 family: participation in multiple cellular processes in eukaryotes. *Cell Signal.* 2005;17(6):675-89.
154. Schitteck B, Sinnberg T. Biological functions of casein kinase 1 isoforms and putative roles in tumorigenesis. *Mol Cancer.* 2014;13:231.

155. Hoekstra MF, Liskay RM, Ou AC, DeMaggio AJ, Burbee DG, Heffron F. HRR25, a putative protein kinase from budding yeast: association with repair of damaged DNA. *Science*. 1991;253(5023):1031-4.
156. Behrend L, Milne DM, Stoter M, Deppert W, Campbell LE, Meek DW, et al. IC261, a specific inhibitor of the protein kinases casein kinase 1-delta and -epsilon, triggers the mitotic checkpoint and induces p53-dependent postmitotic effects. *Oncogene*. 2000;19(47):5303-13.
157. Stoter M, Bamberger AM, Aslan B, Kurth M, Speidel D, Loning T, et al. Inhibition of casein kinase I delta alters mitotic spindle formation and induces apoptosis in trophoblast cells. *Oncogene*. 2005;24(54):7964-75.
158. Brockman JL, Gross SD, Sussman MR, Anderson RA. Cell cycle-dependent localization of casein kinase I to mitotic spindles. *Proc Natl Acad Sci U S A*. 1992;89(20):9454-8.
159. Wang L, Lu A, Zhou HX, Sun R, Zhao J, Zhou CJ, et al. Casein kinase 1 alpha regulates chromosome congression and separation during mouse oocyte meiotic maturation and early embryo development. *PLoS One*. 2013;8(5):e63173.
160. Bozatzi P, Dingwell KS, Wu KZ, Cooper F, Cummins TD, Hutchinson LD, et al. PAWS1 controls Wnt signalling through association with casein kinase 1alpha. *EMBO Rep*. 2018;19(4).
161. Bozatzi P, Sapkota GP. The FAM83 family of proteins: from pseudo-PLDs to anchors for CK1 isoforms. *Biochem Soc Trans*. 2018;46(3):761-71.
162. Fulcher LJ, Bozatzi P, Tachie-Menson T, Wu KZL, Cummins TD, Bufton JC, et al. The DUF1669 domain of FAM83 family proteins anchor casein kinase 1 isoforms. *Sci Signal*. 2018;11(531).
163. Fulcher LJ, He Z, Mei L, Macartney TJ, Wood NT, Prescott AR, et al. FAM83D directs protein kinase CK1alpha to the mitotic spindle for proper spindle positioning. *EMBO Rep*. 2019:e47495.
164. Knippschild U, Kruger M, Richter J, Xu P, Garcia-Reyes B, Peifer C, et al. The CK1 Family: Contribution to Cellular Stress Response and Its Role in Carcinogenesis. *Front Oncol*. 2014;4:96.
165. Wang T, Birsoy K, Hughes NW, Krupczak KM, Post Y, Wei JJ, et al. Identification and characterization of essential genes in the human genome. *Science*. 2015;350(6264):1096-101.
166. Gruss OJ, Wittmann M, Yokoyama H, Pepperkok R, Kufer T, Sillje H, et al. Chromosome-induced microtubule assembly mediated by TPX2 is required for spindle formation in HeLa cells. *Nat Cell Biol*. 2002;4(11):871-9.
167. Scrofani J, Sardon T, Meunier S, Vernos I. Microtubule nucleation in mitosis by a RanGTP-dependent protein complex. *Curr Biol*. 2015;25(2):131-40.
168. Maxwell CA, Keats JJ, Belch AR, Pilarski LM, Reiman T. Receptor for hyaluronan-mediated motility correlates with centrosome abnormalities in multiple myeloma and maintains mitotic integrity. *Cancer Res*. 2005;65(3):850-60.
169. Groen AC, Cameron LA, Coughlin M, Miyamoto DT, Mitchison TJ, Ohi R. XRHAMM functions in ran-dependent microtubule nucleation and pole formation during anastral spindle assembly. *Curr Biol*. 2004;14(20):1801-11.
170. Zhang X, Chen Q, Feng J, Hou J, Yang F, Liu J, et al. Sequential phosphorylation of Nedd1 by Cdk1 and Plk1 is required for targeting of the gammaTuRC to the centrosome. *J Cell Sci*. 2009;122(Pt 13):2240-51.

- 1 171. Zhu H, Fang K, Fang G. FAM29A, a target of Plk1 regulation, controls the partitioning
2 of NEDD1 between the mitotic spindle and the centrosomes. *J Cell Sci.* 2009;122(Pt
3 15):2750-9.
- 4 172. Malik R, Lenobel R, Santamaria A, Ries A, Nigg EA, Korner R. Quantitative analysis of
5 the human spindle phosphoproteome at distinct mitotic stages. *J Proteome Res.*
6 2009;8(10):4553-63.
- 7 173. Malik R, Nigg EA, Korner R. Comparative conservation analysis of the human mitotic
8 phosphoproteome. *Bioinformatics.* 2008;24(12):1426-32.
- 9 174. Martini S, Soliman T, Gobbi G, Mirandola P, Carubbi C, Masselli E, et al. PKCepsilon
10 Controls Mitotic Progression by Regulating Centrosome Migration and Mitotic Spindle
11 Assembly. *Mol Cancer Res.* 2018;16(1):3-15.
- 12 175. Saurin AT, Durgan J, Cameron AJ, Faisal A, Marber MS, Parker PJ. The regulated
13 assembly of a PKCepsilon complex controls the completion of cytokinesis. *Nat Cell Biol.*
14 2008;10(8):891-901.
- 15 176. Fry AM, Bayliss R, Roig J. Mitotic Regulation by NEK Kinase Networks. *Front Cell Dev*
16 *Biol.* 2017;5:102.
- 17 177. O'Regan L, Blot J, Fry AM. Mitotic regulation by NIMA-related kinases. *Cell Div.*
18 2007;2:25.
- 19 178. St-Denis NA, Bailey ML, Parker EL, Vilk G, Litchfield DW. Localization of
20 phosphorylated CK2alpha to the mitotic spindle requires the peptidyl-prolyl isomerase Pin1.
21 *J Cell Sci.* 2011;124(Pt 14):2341-8.
- 22 179. Roth S, Fulcher LJ, Sapkota GP. Advances in targeted degradation of endogenous
23 proteins. *Cell Mol Life Sci.* 2019.
- 24 180. Sakamoto KM, Kim KB, Kumagai A, Mercurio F, Crews CM, Deshaies RJ. Protacs:
25 chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination
26 and degradation. *Proc Natl Acad Sci U S A.* 2001;98(15):8554-9.

Figure Legends:

Figure 1: Anchoring proteins in the regulation of Aurora kinase localisation: **A:** AURKA localises to the centrosomes in a Bora-dependent manner, where Bora acts to facilitate AURKA-dependent activation of PLK1. As M phase progresses, activated PLK1 phosphorylates Bora to promote Bora proteolysis, and AURKA associates with TPX2 to facilitate its localisation to the spindle microtubules. TPX2 serves as an allosteric activator of AURKA. **B:** AURKB forms the so-called Chromosome Passenger Complex (CPC), composed of AURKB and three associated non-enzymatic proteins termed INCENP, Survivin and Borealin. Following Histone H3 phosphorylation at Thr3 by the Ser/Thr kinase Haspin, Survivin mediates recruitment of the CPC to chromatin. At anaphase, Histone H3 is dephosphorylated and the CPC relocates to the central spindle, through binding the kinesin-6 microtubule-binding protein MKLP2. Created with BioRender.com.

Figure 2: The polo-box domain determines the subcellular distribution of PLK1: **A:** Schematic overview of PLK1 domain architecture, highlighting the N-terminal kinase domain, which needs to be activated by an upstream kinase, and the C-terminal Polo-box Domain (PBD), which binds to phosphorylated epitopes on anchoring proteins to promote PLK1 association with the anchoring protein. **B:** PLK1 is activated through phosphorylation of Thr210 by an upstream kinase, principally thought to be the AURKA-Bora complex. The PBD of PLK1 determines the localisation of PLK1 and its recruitment to substrates, by binding to phosphorylated anchoring proteins. Such phosphorylated anchoring proteins are phosphorylated by a priming kinase, most frequently CDK1-Cyclin B or PLK1 itself. **C:** An overview of the centrosome-localised PLK1 anchoring proteins: Gravin and Cep192 simultaneously bind PLK1 and AURKA. Whilst Gravin interacts with PLK1 following priming phosphorylation by CDK1, the Cep192-PLK1 interaction is thought to rely on PLK1-dependent phosphorylation of Cep192. Cenexin is another example of a centrosome-localised CDK1-dependent PLK1 anchoring protein. **D:** An overview of kinetochore-localised PLK1 anchoring proteins: Bub1, CLIP-170 and Dynactin promote PLK1-recruitment to kinetochores in a CDK1-dependent manner. **E:** An overview of central spindle-localised PLK1 anchoring proteins. During metaphase CDK1 activity is high, and CDK1-Cyclin B phosphorylates PRC1 on Thr470 and Thr481 to inhibit the association of PRC1 with PLK1. During anaphase, CDK1 activity is reduced, and PLK1 can phosphorylate PRC1 on Thr602 to promote the PRC1-PLK1 interaction, and concurrent recruitment of PLK1 to the central spindle. MKLP2 is another central spindle protein that anchors PLK1 to the central spindle, following PLK1-dependent phosphorylation of MKLP2 on Ser528. Created with BioRender.com.

Figure 3: KNL1 facilitates the spindle assembly checkpoint by anchoring the mitotic checkpoint complex: **A:** After sensing an unattached kinetochore, the kinase Mps1 phosphorylates MELT repeats on KNL1 to promote downstream assembly of the mitotic checkpoint complex. **B:** Following MELT repeat phosphorylation, Bub1 and Bub3 bind to KNL1, and Bub1 functions to recruit the other Mitotic Checkpoint Complex (MCC) components Mad1, Mad2 and BubR1 to trigger the Spindle Assembly Checkpoint (SAC). The SAC signal inhibits chromosome segregation until all chromosomes are correctly attached and biorientated. Loss of KNL1 leads to mislocalisation of all SAC proteins, with the exception of Mps1. Created with BioRender.com.

Figure 4: Isolating the mitotic kinase activity of a promiscuous kinase: **A:** CK1 α is a Ser/Thr protein kinase involved in many diverse cellular processes. **B:** Selective recruitment of CK1 α to spindle microtubules by FAM83D: In interphase, the FAM83D-HMMR complex cannot associate with CK1 α . During mitosis, the FAM83D-HMMR complex localises to spindle microtubules, and, through as of yet unidentified mechanisms, FAM83D binds CK1 α and recruits it to the mitotic spindle to coordinate proper spindle positioning. CK1 α phosphorylates FAM83D, but the exact relevance of this phosphorylation event is still to be determined. **C:** CK1 α is a pleiotropic kinase with critical roles in both interphase and mitosis. Thus, pan-cellular inhibition or knockdown of CK1 α will affect all of these processes, and specific mitotic effects will be hard to infer. Targeting FAM83D, on the other hand, may present a means to selectively disrupt the mitotic functions of CK1 α , without impacting its other physiological roles. Created with BioRender.com.

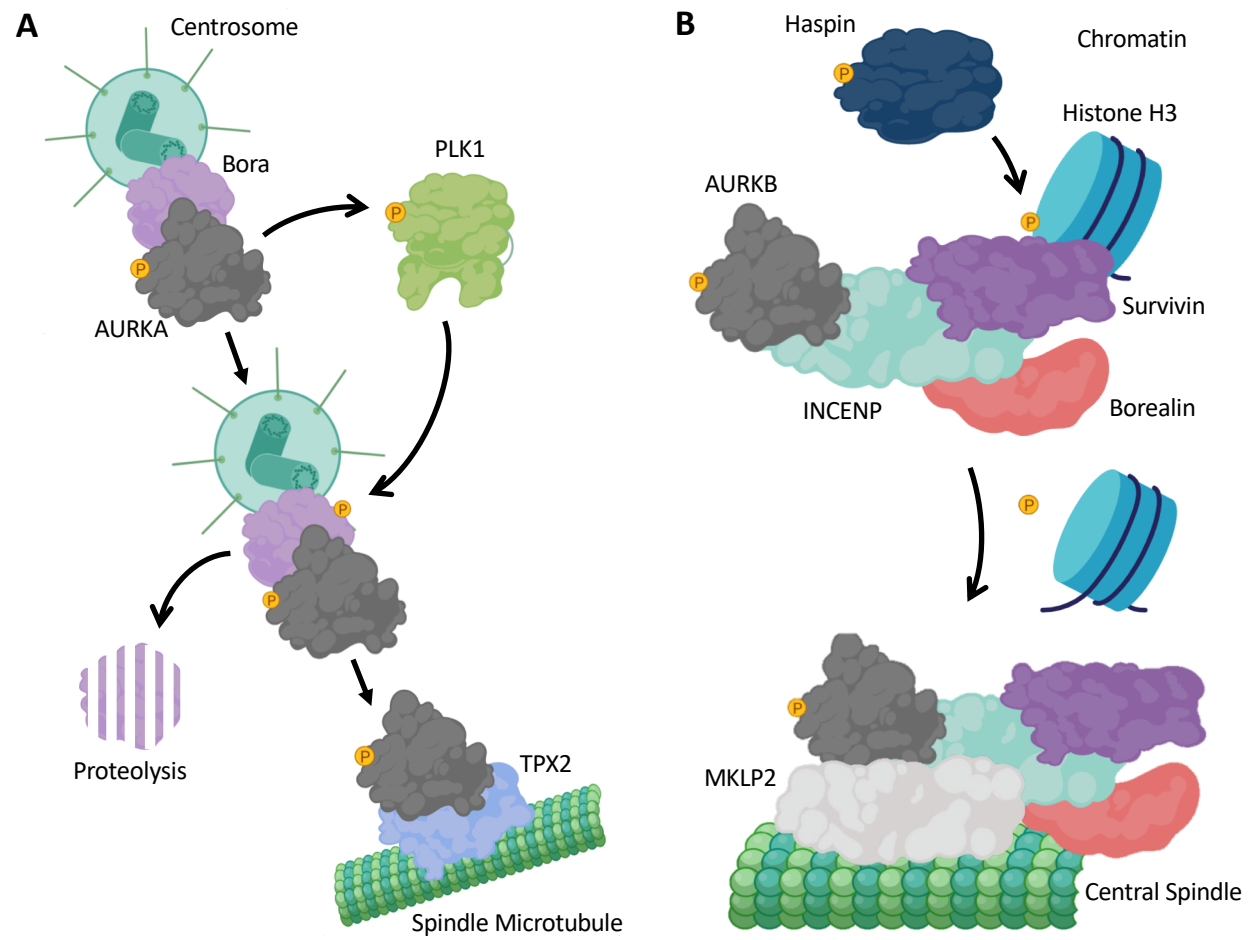


Figure 1

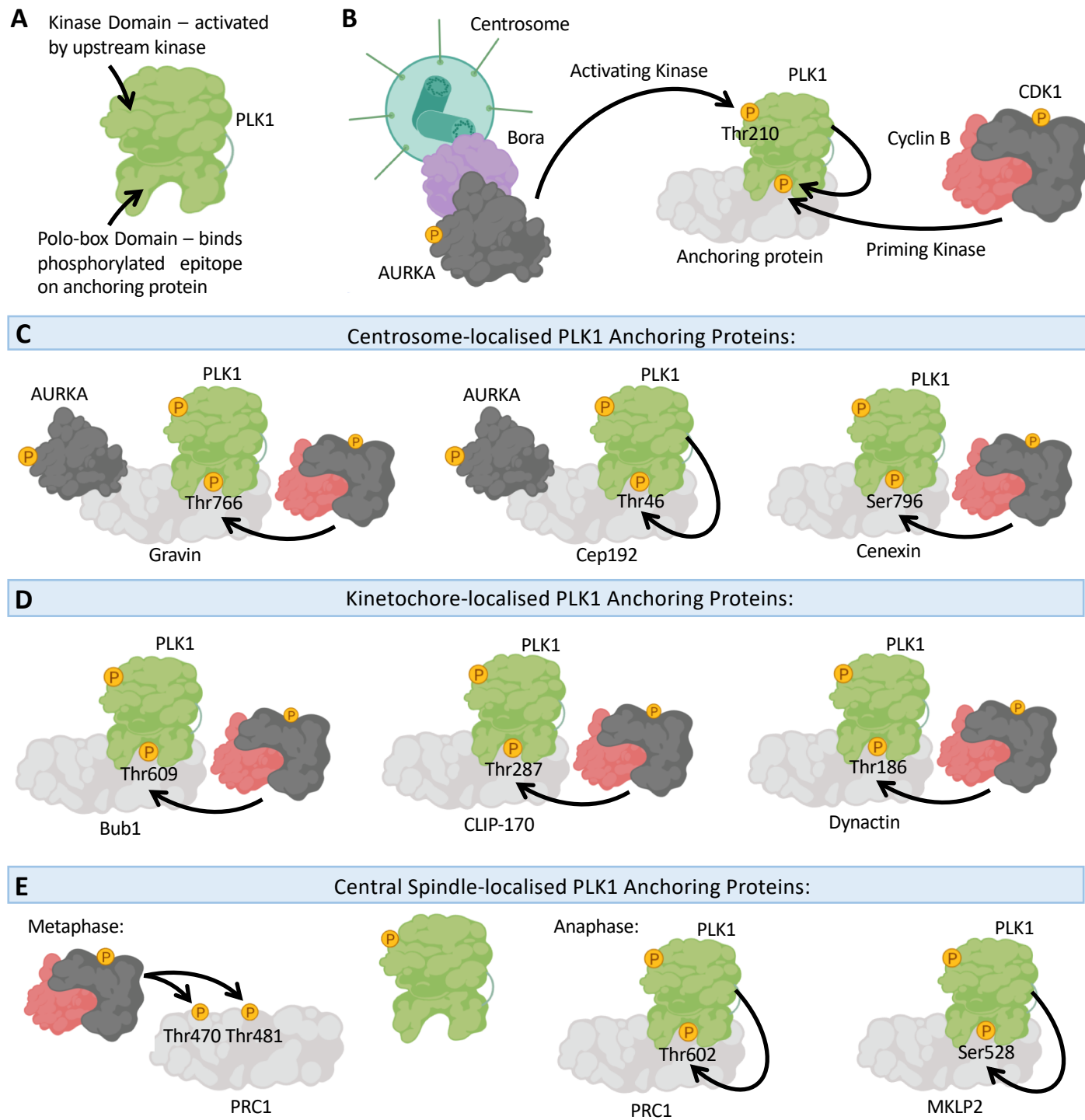


Figure 2

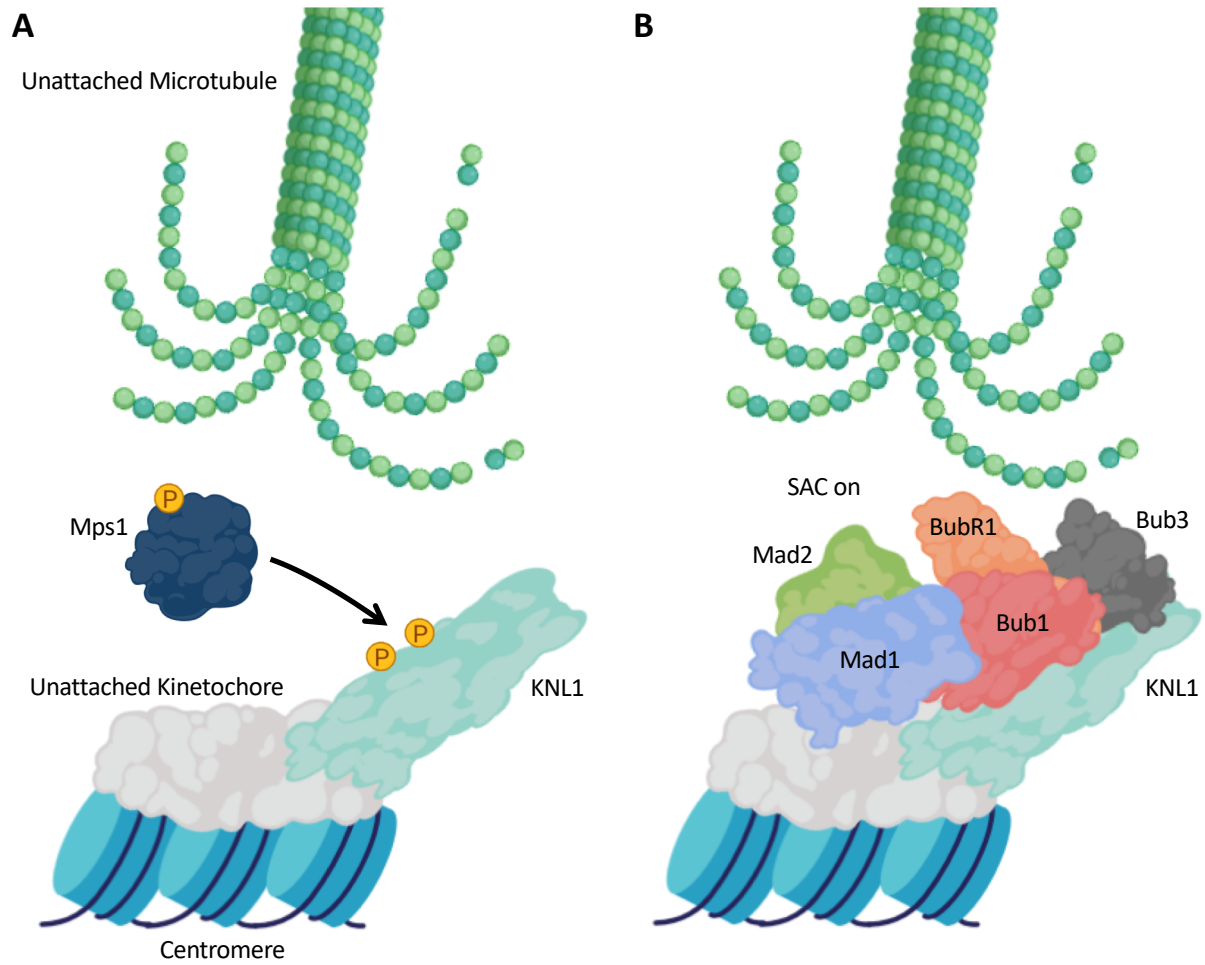


Figure 3

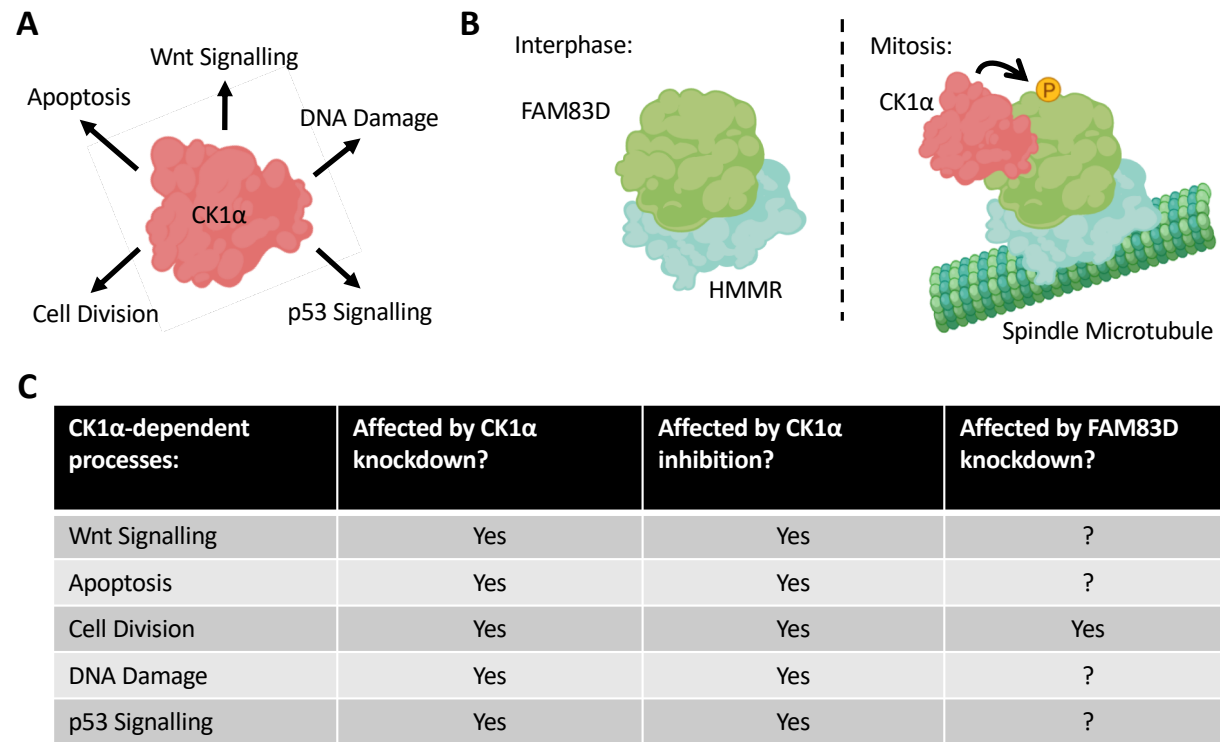


Figure 4

Type of Regulatory Protein	Description
Adaptor Protein	A protein, usually small in size, which serves to link <i>two</i> functional enzymes within a signaling cascade. Adaptor proteins usually exploit protein-protein interaction domains to facilitate their binding to their associated enzymes.
Anchoring/Scaffold Protein	Often used interchangeably, these terms refer to accessory proteins that bind <i>more than two</i> signaling components, and are capable of regulating the activity of that protein complex through various mechanisms, such as the activation or repression of catalytic activity. <i>Anchoring protein</i> is typically used to describe a protein that determines the subcellular localization of the interacting partners, whereas <i>scaffold protein</i> is used preferentially when describing a signalosome assembly platform. For the purpose of this review, use of the term <i>anchoring protein</i> is principally intended to define mitotic kinase interacting proteins that determine the subcellular distribution of the interacting mitotic kinases, but this definition does not limit potential additional roles beyond determination of subcellular distribution.
Docking Protein	Strictly speaking, the term <i>docking protein</i> refers to an accessory protein within a receptor tyrosine kinase signaling pathway that is composed of a <i>membrane-targeting domain</i> , a protein-protein interaction domain, and an extended region containing many Tyr residues for receptor-mediated phosphorylation.